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<p>(21) International Application Number: PCT/US93/01544 (22) International Filing Date: 18 February 1993 (18.02.93) (30) Priority data: 838,509 19 February 1992 (19.02.92) US (71) Applicant: THE STATE OF OREGON acting by and through THE OREGON STATE BOARD OF HIGHER EDUCATION ON BEHALF OF OREGON STATE UNIVERSITY [US/US]; Corvallis, OR 97331 (US). (72) Inventors: DOUGHERTY, William, G. ; 35163 Lillian Drive, Philomath, OR 97370 (US). LINDBO, John, A. ; 2910 N.W. Polk Street, #17, Corvallis, OR 97330 (US).</p>		<p>(74) Agents: POLLEY, Richard, J. et al.; Klarquist, Sparkman, Campbell, Leigh &amp; Winston, One World Trade Center, Suite 1600, 121 S.W. Salmon Street, Portland, OR 97204 (US).  (81) Designated States: CA, JP, NO, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).  Published <i>With international search report.</i></p>
<p>(54) Title: PRODUCTION OF VIRAL RESISTANT PLANTS VIA INTRODUCTION OF UNTRANSLATABLE PLUS SENSE VIRAL RNA</p> <p>(57) Abstract</p> <p>Plants, such as tobacco, are made resistant to potyvirus infection by transformation with vectors which include a gene, derived from a potyvirus, mutated to encode an untranslatable plus sense RNA molecule. Mutagenized potyvirus genes and plant transformation vectors containing these genes are also disclosed.</p>		

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"Production of viral resistant plants via introduction of untranslatable plus sense viral RNA"

#### FIELD OF THE INVENTION

5           This invention is directed to the production of plants with a reduced susceptibility to virus infection.

#### BACKGROUND OF THE INVENTION

10           Plant viruses are responsible for major losses in worldwide crop production. Much effort is directed towards the development of new plant varieties which exhibit increased resistance to viral infection. Until recently such efforts were primarily based on the traditional plant breeding approach, however this approach is often limited by a lack of sources of  
15           resistance within the crop species. The advent of modern molecular biology techniques has facilitated the development of new methods of rendering plant varieties resistant to virus attack that are not limited by a requirement for preexisting resistance genes within a  
20           species.

#### Molecular Approaches

          Many of these molecular approaches are based on the theory of pathogen derived resistance (Sanford and Johnston, 1985). This theory predicts that a "normal"  
25           host (plant) - pathogen (virus) relationship can be disrupted if the host organism expresses essential pathogen derived genes. It has been proposed that host organisms expressing pathogen gene products in excess amounts, at an inappropriate developmental stage, or in  
30           a dysfunctional form may disrupt the normal replicative cycle of the pathogen and result in an attenuated or aborted infection of the host.

          Two approaches typify this pathogen derived resistance: coat protein mediated resistance and  
35           antisense RNA expression. It has been demonstrated that transgenic plants expressing a plant virus coat protein can be resistant to infection by the homologous virus. This coat protein mediated resistance has been

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demonstrated for several virus groups. While the mechanism of this resistance is not yet fully understood, it has been suggested that the presence of the plant synthesized coat protein prevents the removal of the protein coat (uncoating) of an invading virus and/or virus movement within the infected plant, leading to resistance.

Plants which express an RNA molecule which is complementary to plus sense RNA species encoded by the virus may show a decreased susceptibility to infection by that virus. Such a complementary RNA molecule is termed antisense RNA. It is thought that the plant encoded antisense RNA binds to the viral RNA and thus inhibits its function.

#### Potyviruses

The Potato Virus Y, or potyvirus, family represents a large number of plant viral pathogens which collectively can infect most crop species including both monocotyledonous and dicotyledonous plants. Potyvirus infection can induce a variety of symptoms including leaf mottling, seed and fruit distortion and can severely compromise crop yield and/or quality (Hollings and Brunt, 1981).

Potyviruses have a single-strand plus sense RNA of circa 10,000 nucleotides which has a viral encoded protein linked to the 5' end and a 3' polyadenylate region. A single open reading frame codes for a 351 kDa polyprotein which is proteolytically processed into mature viral gene products. The RNA is encapsidated by approximately 2,000 copies of a coat protein monomer to form a virion. This capsid protein is encoded by the sequence present at the 3' end of the large open reading frame.

Potyviruses can be transmitted by aphids and other sap feeding insects and in some instances can also be transmitted in the seeds of infected plants. Replication of the viral RNA is thought to occur in the cytoplasm of infected plant cells after uncoating. The

replication mechanism involves both translation of the plus sense RNA to yield viral gene products (which include a replicase and a proteinase) and also the synthesis of a minus sense RNA strand. This minus sense strand then acts as a template for the synthesis of many plus sense genomes which are subsequently encapsidated in coat protein to yield infectious mature "virions," thus completing the replicative cycle of the virus.

Experiments have been reported in which transgenic plants expressing the coat protein gene of a potyvirus show a reduced susceptibility to virus infection (Lawson et al. 1990; Ling et al. 1991; Stark and Beachy 1989).

#### SUMMARY OF THE INVENTION

The disclosed invention concerns a method of producing plants with a decreased susceptibility to virus infection. This is achieved by transforming plants with a DNA molecule which includes a gene derived in part from the genome of a plant virus. This gene is specifically constructed to produce an untranslatable version of a plus sense RNA molecule required for viral replication. Thus, expression of the gene within the plant causes the production of this non-functional molecule which then inhibits viral replication within the plant, rendering the plant resistant to viral infection.

In particular, invention provides an alternative and novel approach to rendering plants resistant to potyvirus infection.

Plants are transformed with a gene construct engineered to express an untranslatable form of the plus sense RNA which encodes the coat protein of a potyvirus.

In the case of Tobacco Etch Virus (TEV), it is demonstrated that tobacco plants transformed with such a gene construct accumulate the untranslatable plus sense RNA but do not produce detectable levels of the coat protein. It is further shown that these plants are resistant to TEV infection. It is also shown that

tobacco cells expressing this untranslatable plus sense RNA do not support TEV replication, unlike control tobacco cells and also unlike tobacco cells which are engineered to express the plus sense translatable RNA and which, as a result, accumulate TEV coat protein. Although the exact mechanism is unknown, it is proposed that the untranslatable plus sense RNA inhibits viral replication by binding to the minus sense RNA and preventing the minus sense RNA from functioning in the replication cycle.

It is believed that this approach will be applicable to other potyviruses, to genes other than the coat protein gene and to other plus sense RNA virus families. It is also believed that this means of inhibiting gene function is applicable to other biological systems, including mammalian viruses.

#### DESCRIPTION OF DRAWINGS

Fig. 1 represents the nucleotide sequence of the Tobacco Etch Virus genome and its deduced amino acid sequence, according to Allison et al. (1986). The nucleotide sequence of the plus sense strand of the DNA inserts is given. The first nucleotide (N) could not be determined unequivocally. The predicted amino acid sequence of the large ORF of reading frame three of the viron sense RNA is presented in the nucleotide sequence. This sequence is also set forth in SEQ ID No. 1 of the enclosed sequence listing. The termination codon at the end of the large ORF is marked with a \*. The putative cleavage site between the large (54,000 Mw) nuclear inclusion protein and the capsid protein is indicated by the arrow. Oligonucleotide primer binding sites are underlined and labeled.

Fig. 2 is a schematic representation of the construction of pTC:FL, utilized in construction of transformation vectors for the invention. Restriction endonuclease sites were introduced into pTL 37/8595 at positions A, B and C in the diagram. Following these nucleotide changes the mutated pTL 37/8595 was digested

with the restriction enzyme *Nco*I, the DNA fragment delineated by the restriction enzyme sites at B and C was removed, and the plasmid religated to generate pTC:FL. pTC:FL contains the Tobacco Etch Virus (TEV) coat protein nucleotide sequence flanked by *Bam*HI restriction sites and the TEV 5' and 3' untranslated sequences (UTS). T7 and SP6 promoters are also shown. Abbreviations used in this diagram are as follows: T7, T7 RNA polymerase promoter sequence; SP6, SP6 RNA polymerase promoter sequence; ori, origin of replication; M13 ori, bacteriophage M13 single-stranded origin of replication; amp<sup>r</sup>,  $\beta$ -lactamase gene. Lightly stippled areas are TEV 5' and 3' untranslated sequences; solid black area, TEV genome cDNA nucleotides 144 to 200; striped area, a portion of the TEV N1b gene (TEV nt 8462-8517); heavily stippled areas, cDNA of TEV CP nucleotide sequence (TEV nt 8518-9309).

Fig. 3 is a schematic representation of the forms of the Tobacco Etch Virus coat protein gene inserted into tobacco in the invention. All constructs contained the enhanced CaMV 35S (Enh 35S) promoter, CaMV 35S 5' untranslated sequence (UTS) of 50 bp and the CaMV 35S 3' UTS/polyadenylation site of 110 bp. The nomenclature used to describe the transgenic plant lines is presented along with the gene products produced in those plant lines (far right column). Abbreviations are as follows: 35S, transgenic plants containing the CaMV 35S promoter and 5' and 3' UTS only; FL, transgenic plants containing the transgene coding for full-length, AS and RC transgenic plants contain the transgene expressed as an antisense form of the TEV CP gene, or an untranslated sense form of the TEV CP gene, respectively. Stippled areas represent various forms of the TEV CP nucleotide sequence.

Fig. 4 is a graphic representation of the appearance of systemic symptoms in plants infected with Tobacco Etch Virus showing responses of control plants and transformed plants generated as described in the

invention. Ten B49 (wild type) plants and ten R2 plants of transgenic plant lines 35S #4, FL #3, FL #24, homozygous for the inserted TEV gene, were mechanically inoculated with 50  $\mu$ l of 1:10 dilution of infected plant sap (A). Twenty B49 plants and 20 R1 plants of lines AS #3 and RC #5 were mechanically inoculated with 50  $\mu$ l of 5  $\mu$ g/ml TEV (B). Plants were examined daily for the appearance of systemic symptoms. Plants were evaluated daily, and any plant displaying systemic symptoms (attenuated or wild-type) were recorded as symptomatic.

#### SEQUENCE LISTING

The attached sequence listing sets forth nucleotide sequences relevant to the present invention.

SEQ ID No. 1 is the complementary DNA sequence corresponding to the Tobacco Etch Virus Genome.

SEQ ID No. 2 is the nucleotide sequence of the modified Tobacco Etch Virus coat protein gene present in pTC:FL.

SEQ ID No. 3 is the nucleotide sequence of the modified Tobacco Etch Virus coat protein gene present in pTC:RC.

SEQ ID No. 4 is the nucleotide sequence of the modified Tobacco Etch Virus coat protein gene present in pTC:AS. It is the inverse complement of SEQ ID No. 2.

#### DETAILED DESCRIPTION

The present invention relates to genetically engineered plants which are transformed with a DNA molecule encoding an untranslatable plus sense RNA molecule.

#### Definition of Terms

Susceptible plant: A plant that supports viral replication and displays virus-induced symptoms.

Resistant plant: A plant wherein virus-induced symptoms are attenuated and virus replication is attenuated.

Plus sense RNA (and sense RNA): That form of an RNA which can serve as messenger RNA.

Minus sense RNA: That form of RNA used as a template for plus sense RNA production.

Antisense RNA: RNA complementary to plus sense RNA form.

5         $R_0$  generation: Primary transformants.

$R_1$  generation: Progeny of primary transformants.

$R_2$  generation: Second generation progeny of  $R_0$  generation (i.e., progeny of  $R_1$  generation).

10        A gene derived in part from a plant virus RNA molecule: At least the portion of the gene encoding the untranslatable RNA molecule is derived from a plant virus RNA molecule.

#### GENERAL DESCRIPTION

15        An untranslatable plus sense RNA molecule is encoded by a gene located on the DNA molecule. The gene comprises DNA derived from a plant virus RNA genome and also DNA from heterologous sources. The DNA from heterologous sources includes elements controlling the  
20        expression of the virus-derived DNA sequences. The DNA sequence of the gene is specifically altered so as to render the RNA molecule transcribed from the gene untranslatable. The presence of this untranslatable plus sense RNA within the cells of the transformed plant  
25        reduces the susceptibility of the plant to viral infection.

More particularly, the portion of the gene which comprises DNA from a plant virus has been derived from a potyvirus. Plants transformed with the DNA  
30        molecule containing the gene are less susceptible to infection by potyviruses. Most specifically, the DNA from the potyvirus source has been derived from the coat protein gene of Tobacco Etch Virus and transformed plants are resistant to infection by Tobacco Etch Virus.  
35        Plants which can be made resistant to potyvirus infection include, but are not limited to, tobacco.

Accordingly, the present invention provides a method for genetically engineering plants by insertion,

into the plant genome, a DNA construct containing a recombinant gene derived from a potyvirus genome such that the engineered plants display resistance to the potyvirus.

5 In accordance with one aspect of the present invention, genetically transformed plants which are resistant to infection by a plant potyvirus are produced by inserting into the genome of the plant a DNA sequence which causes the production of an untranslatable coat  
10 protein RNA of the potyvirus.

In accordance with another aspect of the present invention, a DNA sequence is provided to function in plant cells to cause the production of an untranslatable plus sense RNA molecule. There has also  
15 been provided, in accordance with yet another aspect of the present invention, bacterial and transformed plant cells that contain the above-described DNA. In accordance with yet another aspect of the present invention, a differentiated tobacco plant has been  
20 provided that comprises transformed tobacco cells which express the untranslatable coat protein RNA of Tobacco Etch Virus and which plants exhibit resistance to infection by Tobacco Etch Virus.

Other features and advantages of the present  
25 invention will become apparent from the following description. It should be understood, however, that the detailed description and the specific examples, while indicating preferred embodiments of the invention, are given by way of illustration only, since various changes  
30 and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

A mechanism by which an untranslatable plus sense RNA molecule, such as described in the current  
35 invention can function to inhibit the normal biological function of a minus sense RNA molecule is proposed. One skilled in the art will recognize that the novel approach described herein is not limited to the specific

experimental example given and will appreciate the wider potential utility of the invention.

The expression of a plant gene which exists in double-stranded DNA form involves transcription of messenger RNA (mRNA) from one strand of the DNA by RNA polymerase enzyme, and the subsequent processing of the mRNA primary transcript inside the nucleus. This processing involves a 3' nontranslated region which causes polyadenylate nucleotides to be added to the 3' end of the viral RNA. Transcription of DNA into mRNA is regulated by a region of DNA usually referred to as the "promoter." The promoter region contains a sequence of bases that signals RNA polymerase to associate with the DNA and to initiate the transcription of mRNA using one of the DNA strands as a template to make a corresponding strand of RNA.

A number of promoters which are active in plant cells have been described in the literature. Promoters which are known or are found to cause transcription of viral RNA in plant cells can be used in the present invention. Such promoters may be obtained from plants or viruses and include, but are not limited to, the CaMV 35S promoter. As described below, it is preferred that the particular promoter selected should be capable of causing sufficient expression to result in the production of an effective amount of untranslatable plus sense RNA to render the plant substantially resistant to virus infection. The amount of untranslatable plus sense RNA needed to induce resistance may vary with the plant type. Accordingly, while the 35S promoter is preferred, it should be understood that this promoter may not be the optimal one for all embodiments of the present invention. Furthermore, the promoters used in the DNA constructs of the invention may be modified, if desired, to affect their control characteristics. DNA sequences have been identified which confer regulatory specificity on promoter regions. For example, the small subunit of the ribulose bis-phosphate carboxylase (ss

RUBISCO) gene is expressed in plant leaves but not in root tissues. A sequence motif that represses the expression of the ss RUBISCO gene in the absence of light, to create a promoter which is active in leaves but not in root tissue, has been identified. This and/or other regulatory sequence motifs may be ligated to promoters such as the CaMV 35S promoter to modify the expression patterns of a gene. Chimeric promoters so constructed may be used as described herein. For purposes of this description, the phrase "CaMV 35S promoter" will therefore include all promoters derived by means of ligation with operator regions, random or controlled mutagenesis, as well as tandem or multiple copies of enhancer elements, and the like.

The 3' nontranslated region of genes which are known or are found to function as polyadenylation sites for viral RNA in plant cells can be used in the present invention. Such 3' nontranslated regions include, but are not limited to, the 3' transcribed, nontranslated region of the CaMV 35S gene and the 3' transcribed, nontranslated regions containing the polyadenylation signals of the tumor-inducing (TI) genes of *Agrobacterium*, such as the tumor morphology large (tml) gene. For purposes of this description, the phrase "CaMV 35S 3' nontranslated region" will therefore include all such appropriate 3' nontranslated regions.

The DNA constructs of the disclosed embodiment contain, in double-stranded DNA form, a portion of a cDNA version of the single-stranded RNA genome of TEV. In potyviruses, including TEV, the viral genome includes genes encoding the coat protein, a replicase enzyme and a proteinase. The disclosed embodiment utilizes the region of the genome encoding the coat protein gene. In considering the present invention and the evidence for the proposed mechanism by which an untranslatable plus sense RNA molecule can inhibit viral replication, those skilled in the art will recognize that other portions of a potyvirus genome could be substituted for the coat

protein gene. Furthermore, it will be apparent that suitable genomic portions are not limited to complete gene sequences.

A disclosed embodiment of the invention  
5 utilizes a double-stranded complementary DNA (cDNA)  
derived from the region of the TEV genome encoding the  
coat protein gene. To the 5' end of this cDNA is  
ligated the CaMV 35S promoter and CaMV 35S RNA 5'  
nontranslated region. To the 3' end is ligated the CaMV  
10 35S 3' nontranslated region. These 5' and 3' sequences  
are present to cause transcription of the gene in plant  
cells by the cellular enzyme RNA polymerase to produce  
an RNA molecule of sequence corresponding to the  
sequence of the coat protein cDNA sequence. Ordinarily,  
15 such an RNA would then be translated by ribosomes which  
would synthesize a protein of amino acid sequence  
specified by the nucleotide sequence of the RNA  
molecule. Particular amino acids are specified by  
nucleotide triplets termed codons. Codons which  
20 stipulate translation initiation and termination are  
also present in DNA and RNA sequences. The current  
invention relates to RNA molecules which are  
untranslatable by ribosomes. In the preferred  
embodiment the sequence of the TEV cDNA encoding the  
25 coat protein is mutated by a standard in vitro  
mutagenesis technique to produce a frameshift mutation  
early in the coat protein structural gene immediately  
followed by three translation termination signal codons.  
These mutations do not affect the ability of RNA  
30 polymerase to transcribe an RNA molecule from the cDNA  
but prevent translation of the transcribed RNA by  
ribosomes. Those skilled in the art will recognize that  
for the disclosed gene and for other genes, DNA  
sequences can be altered in other ways to cause the DNA  
35 to encode an untranslatable plus sense RNA molecule.  
Thus the disclosed invention is not limited to the  
mutations disclosed.

A disclosed embodiment utilizes a cDNA encoding the coat protein gene of TEV, mutated so as to encode an untranslatable plus sense RNA. It will be obvious to one skilled in the art that further sequence alteration of the cDNA molecule could be used to confer additional features on the untranslatable plus sense RNA molecule. Additional features include those which would result in increased viral resistance of plants transformed with the cDNA molecule encoding an untranslatable plus sense RNA. The inclusion of a ribozyme sequence which causes the RNA catalyzed destruction of the target RNA molecule would constitute one such additional feature. Suitable ribozyme sequences are known, as discussed in Tabler and Tsagris (1991).

A DNA construct in accordance with the present invention is introduced, via a suitable vector and transformation method as described below, into plant cells and plants transformed with the introduced DNA are regenerated. Various methods exist for transforming plant cells and thereby generating transgenic plants. Methods which are known or are found to be suitable for creating stably transformed plants can be used in this invention. The choice of method will vary with the type of plant to be transformed; those skilled in the art will recognize the suitability of particular methods for given plant types. Suitable methods may include, but are not limited to: electroporation of plant protoplasts; liposome mediated transformation; polyethylene mediated transformation; transformation using viruses; microinjection of plant cells; microprojectile bombardment of plant cells and *Agrobacterium tumefaciens* (AT) mediated transformation. The latter technique is the method of choice for the disclosed preferred embodiment of the present invention.

In an embodiment of the current invention, the DNA sequences comprising the CaMV 35S promoter and CaMV 35S nontranslated 3' region and the mutated cDNA encoding an untranslatable plus sense RNA derived from

the TEV coat protein gene are combined in a single cloning vector. This vector is subsequently transformed into AT cells and the resultant cells are used to transform cultured tobacco cells.

5                    Vectors suitable for the AT mediated transformation of plants with the DNA of the invention are disclosed. It will be obvious to one skilled in the art that a range of suitable vectors is available, including those disclosed by Bevan (1983),  
10                    Herrera-Estrella (1983), Klee (1985) and EPO publication 12,516 (Schilperoort et al.). Suitable vectors are available on a commercial basis from Clontech (Palo Alto, CA) and Pharmacia LKB (Pleasant Hill, CA) and other sources.

15                    Following the transformation of plant cells and regeneration of transformed plants with the DNA molecules as described, regenerated plants are tested for increased virus resistance. Plants are preferably exposed to the virus at a concentration within a range  
20                    where the rate of disease development correlates linearly with virus concentration. Methods for virus inoculation are well known to those skilled in the art and are reviewed by Kado and Agrawai (1972). One such method includes abrading a leaf surface with an aqueous  
25                    suspension containing an abrasive material such as carborundrum and virus or dusting leaves with such an abrasive material and subsequently applying the virus onto the leaf surface. A virus suspension can be  
30                    directly inoculated into leaf veins or alternatively plants can be inoculated using insect vectors. The virus suspension may comprise purified virus particles, or alternatively, sap from virus infected plants may be utilized.

35                    Transformed plants are then assessed for resistance to the virus. The assessment of resistance or reduced susceptibility may be manifest in different ways dependant on the particular virus type and plant type. Those skilled in the art will realize that a

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comparison of symptom development on a number of inoculated untransformed plants with symptom development on similarly inoculated transformed plants will provide a preferred method of determining the effects of transformation with the specified DNA molecule on plant resistance. Symptoms of infection include, but are not limited to leaf mottling, chlorosis and etching. Plants showing increased viral resistance may be recognized by delay in appearance of such symptoms or attenuation or total lack of such symptoms.

#### Example

Work with tobacco plants and the Tobacco Etch Virus (TEV) is illustrative of the invention.

#### Construction of gene encoding untranslatable plus sense RNA molecule.

The Highly Aphid Transmissible (HAT) isolate of Tobacco Etch Virus (TEV) was obtained from Dr. Tom Pirone (University of Kentucky) and maintained in *Nicotiana tabacum* (Burley 21). The virus was purified from *Nicotiana tabacum* (Burley 21) 20 to 30 days following inoculation. Viral purification and RNA isolation procedures have been described (Dougherty and Hiebert (1980a). Complementary DNA (cDNA) was synthesized, made double-stranded and inserted into the bacterial plasmid pBR322 as described by Allison et al. (1985a, 1985b, 1986), herein incorporated by reference. cDNA synthesis was accomplished as follows: Purified viral RNA primed with oligo(dT<sub>12-18</sub>) served as a template for single-strand cDNA synthesis by reverse transcriptase. Following the addition of homopolymeric tracts of deoxycytidine 5' monophosphate, second-strand synthesis, primed with oligo(dG<sub>12-18</sub>), was completed with DNA polymerase I. SalI and EcoRI linkers were ligated to the double-stranded cDNA and inserted into the bacterial plasmid pBR322 (Kurtz and Nicodemus 1981). The resulting cDNA clones were screened by colony hybridization (Hanahan and Meselson 1980) with oligo(dT<sub>12-18</sub>) primed, <sup>32</sup>P-labeled single-stranded TEV

cdNA. Plasmid DNA was isolated from colonies which hybridized with the probe, and the *SalI/EcoRI* cdNA inserts were sized by electrophoresis in a 0.8% (w/v) agarose gel using a horizontal water-cooled gel apparatus.

The *SalI/EcoRI* inserts from the recombinant molecules were isolated from an agarose gel with NA45 membrane (Schleicher & Schuell, Keene, NH) according to the manufacturer's protocol. The following restriction enzymes were used either alone or in combination to digest the isolated cdNA insert: *HindIII*, *XhoI*, *AluI*, *HaeIII*, *RsaI*, *Sau3A*, and *TaqI*. Restriction enzyme digestion products were inserted into the DNA of an appropriate M13 bacteriophage (Messing 1983) selected for the presence of corresponding polylinker restriction sites, and their nucleotide sequences were determined by dideoxy chain termination.

Plasmid pTL 37/8595 (Carrington and Dougherty 1987; Carrington et al. 1987, herein incorporated by reference) contains a cdNA copy of the genomic sequence of HAT TEV corresponding to nucleotides (nt) 1-200 and nt 8462-9495 (Fig. 2). (Numbering of the TEV genome nucleotides is according to that presented in Allison et al. 1986). The nucleotide sequence and deduced amino acid sequence of the Tobacco Etch Virus genome and the numbering system utilized by Allison et al. (1986) and herein is shown in Fig. 1 and SEQ ID No. 1 in the attached sequence listing. The first and last codons of the coat protein (CP) coding region in the TEV genome are nt 8518-8520 (encoding the amino acid serine) and 9307-9309 (opal stop codon) respectively. pTL 37/8595 was subject to *in vitro* site-directed mutagenesis as described by Taylor et al. (1985a, 1985b) herein incorporated by reference. In all cases, nucleotide changes were confirmed by dideoxy-nucleotide sequencing (Sanger et al. 1977).

TEV nt 9312-9317 were first mutated (Fig. 2) to generate a *BamHI* restriction site (GGATCC). TEV nt

8516-8521 were then altered to generate an *Nco*I site (CCATGG), changing the first codon of the TEV CP coding region from AGT (Ser), to ATG (Met). A single oligonucleotide was then used to mutate TEV nt 133-138 to a *Bam*HI restriction site (GGATCC), nt 143-148 to an *Nco*I restriction site (CCATGG) and nt 142 to a deoxyadenylate residue. These mutations generated an *Nco*I site centered on the first codon of the TEV ORF and in a good translational start context as described by Kozak (1984). Digestion of the resulting plasmid with the restriction enzyme *Nco*I; removing TEV nt # 143-200/8462-8516, and religation generated plasmid pTC:FL. pTC:FL contained only the TEV CP gene flanked by *Bam*HI restriction sites and TEV 5' and 3' untranslated sequences (see Fig. 2). The nucleotide sequence of the TEV CP gene in pTC:FL produced by this mutagenesis scheme is shown in SEQ ID No. 2 in the attached sequence listing.

Plasmid pTC:RC (RNA Control, producing untranslatable plus sense RNA) was generated by insertion of a single deoxythymidylate residue after TEV nt 8529, and point mutations of TEV nt 8522 (G to C), 8534 (C to A), 8542 (G to A), and 8543 (A to G) to create a frameshift mutation immediately followed by three stop codons. An *Nhe*I restriction site (GCTAGC) was simultaneously generated, for screening purposes, at nt 8539-8544. The nucleotide sequence of the TEV CP gene in pTC:RC produced by this mutagenesis scheme is shown in SEQ ID No. 3 in the attached sequence listing.

All plasmids described above were linearized with *Hind*III, transcribed with T7 RNA polymerase (Melton et al. 1984), and translated in a rabbit reticulocyte lysate containing <sup>35</sup>S Methionine (Dougherty and Hiebert 1980a). Radiolabeled translation products were analyzed by electrophoretic separation on a 12.5% acrylamide gel containing SDS (Laemmli 1970) and detected by autoradiography. Transcripts of plasmid pTC:RC produced

no detectable protein products, while transcripts from pTC:FL produced proteins of the expected sizes.

The various forms of the CP nucleotide sequence were then inserted as *Bam*HI cassettes into the plant expression vector pPEV (see below and Fig. 3).

The full length TEV CP open reading frame of pTC:FL was inserted in the reverse orientation to make the antisense (AS) construct pTC:AS. The nucleotide sequence of the TEV CP gene in pTC:AS is shown in SEQ ID No. 4 in the attached sequence listing.

#### Transformation Vector Construction

Construction of pPEV. The vector pPEV is part of a binary vector system for *Agrobacterium tumefaciens* mediated plant cell transformation. Plasmid pPEV was constructed from the plasmids pCGN 2113 (Calgene), pCIB 710 and pCIB 200 (Ciba Geigy Corp.). pCGN 2113 contains the "enhanced" Cauliflower Mosaic Virus (CaMV) 35S promoter (CaMV sequences -941 to 90/-363 to +2, relative to the transcription start site) in a pUC derived plasmid backbone. pCIB 710 has been described (Rothstein et al. 1987) and pCIB 200 is a derivative of the wide host range plasmid pTJS 75 (Schmidhauser and Helinski 1985) which contains left and right *A. tumefaciens* T37 DNA borders, the plant selectable NOS/NPT II chimeric gene from the plasmid Bin 6 (Bevan 1984) and part of a pUC polylinker. The small *Eco*RI-*Eco*RV DNA fragment of pCIB 710 (Rothstein et al. 1987) was ligated into *Eco*RI-*Eco*RV digested pCGN 2113. This regenerated the enhanced CaMV 35S promoter (Kay et al. 1987) of pCGN 2113 and introduced the CaMV 35S 5' and 3' untranslated sequences into pCGN 2113. The CaMV 35S promoterterminator cassette of the resulting plasmid was isolated as an *Eco*RI-*Xba*I DNA fragment and ligated into *Eco*RI-*Xba*I digested pCIB 200 to generate pPEV. CP nucleotide sequences from pTC:FL, pTC:RC, and pTC:AS were cloned as *Bam*HI cassettes into *Bam*HI digested pPEV and orientation of inserts confirmed by digestion with appropriate restriction endonucleases.

### Transformation and Regeneration of Tobacco

pPEV plasmids containing TEV CP ORFs were mobilized from *E. coli* HB101 into *A. tumefaciens* A136 containing plasmid pCIB 542 (Ciba Geigy), using the  
5 helper plasmid pRK 2013 in *E. coli* HB101 and the tri-parental mating system of Ditta et al. (1980). Plasmid pCIB 42 supplied *vir* functions necessary for T-DNA transfer.

10 Leaf discs of *Nicotiana tabacum* cv Burley 49 were transformed and whole plants regenerated according to Horsch et al. (1985). Transformed tissue was selected by culturing callus on MS plates (Murashige and Skoog 1962) containing 1 µg/ml 6-benzylaminopurine  
15 (Sigma Corp.), 01 µg/ml α-naphthaleneacetic acid (Sigma Corp.), 500 µg/ml carbenicillin and 100 µg/ml Kanamycin sulfate (Sigma Corp.). Shoots were rooted on MS plates containing 500 µg/ml carbenicillin and 100 µg/ml  
20 kanamycin sulfate, and plantlets were transplanted into soil and transferred directly into the greenhouse approximately 2-3 weeks after rooting.

R0, R1 and R2 generation plants were screened by western and/or northern blot analyses. R2 seed (ca. 100 seeds per R2 plant) was screened for the kanamycin-resistant phenotype (kan<sup>r</sup>) by surface sterilizing seed in  
25 10% bleach for 5 min., washing twice in sterile water and germinating on MS plates containing 100 µg/ml kanamycin sulfate. R2 seed lines which were 100% kanamycin resistant were screened by western blot analysis for expression of TEV coat protein. Those  
30 transgenic plant lines generated and their nomenclature are presented in Fig. 3.

### Molecular Analyses of Transgenic Plants

Transgenic tobacco plants were analyzed by western and northern blot analyses to determine the  
35 nature of protein and RNA products produced respectively. Total RNA samples isolated from the various transgenic lines were analyzed in northern blot hybridization studies. Total nucleic acids were

isolated from tissue and RNA precipitated with LiCl as described by Verwoerd et al. (1989). RNAs were electrophoretically separated on 1.2% agarose gels containing 6% (v/v) formaldehyde and transferred to nitrocellulose. Prehybridization and hybridization conditions were as described in Sambrook et al. (1989). Strand specific riboprobes were generated from SP6 or T7 DNA dependent RNA polymerase transcription reactions of pTL 37/8595 linearized with the restriction enzymes Asp718 (Boehringer Mannheim, Indianapolis, IN) or HindIII, respectively, using  $\alpha$ -labelled  $^{32}\text{P}$ -CTP ribonucleotide and suggested procedures (Promega, Madison, WI).

An RNA transcript of approximately 1,000 nt was expected with all transgenic plant lines. Such a TEV CP transcript was detected in CP expressing plant lines by using a minus sense riboprobe containing the TEV CP sequence. A similar transcript was detected in AS plants by using a plus sense riboprobe containing the TEV CP sequence. The transcript in the RC line, while detected with a minus sense riboprobe, may have migrated as a slightly larger (ca 1,100-1,200 nt) RNA species, possibly due to termination at an alternately selected site and/or a longer poly-A tail on the transcript. Differing levels of CP transcript accumulation were observed among different transgenic plant lines. Transgenic plant lines expressing the coat protein of TEV were identified by western blot analysis using polyclonal antisera to TEV CP. Tissue samples of regenerated plants were ground in 10 volumes of 2X Laemmli (Tris-glycine) runner buffer (Laemmli 1970) and clarified by centrifugation in a microcentrifuge for 10 min. at 10,000xg. Protein concentration was estimated by the dye binding procedure of Bradford (1976) using BSA as a standard. Protein samples (50  $\mu\text{g}$  total protein) were separated on a 12.5% polyacrylamide gel containing SDS and subjected to the immunoblot transfer procedures described by Towbin et al. (1979). Anti-TEV

coat protein polyclonal primary antibodies, alkaline phosphatase conjugated secondary antibodies and the chromogenic substrates NBT (para-nitro blue tetrazolium chloride) and BCIP (5-bromo-4-chloro-3-indoyl phosphate para-toluidine salt) were used to detect bound antigen.

Coat protein products produced in FL plants were stable and accumulated to different levels in individual transgenic plant lines. It was estimated by western blot analysis that between 0.01% to 0.001% of total extracted protein was TEV CP.

#### Assessment of Resistance to TEV

Eight-week-old (circa 15 cm tall) R1 and R2 plants were inoculated with either purified virus preparations or infected plant sap. Inoculum was applied with sterile, premoistened cotton swabs. Infected plant sap inoculum was prepared by grinding TEV-infected *N. tabacum* Burley 21 leaf tissue (2 weeks postinoculation) in carborundum and 50 mM sodium phosphate buffer (pH 7.8) at a ratio of 1gm:02gm:10mls, respectively, and filtering the homogenate through cheesecloth. TEV virions were purified as described by Dougherty and Hiebert (1980b). One leaf per plant was dusted lightly with carborundum (320 grit) and inoculated at two interveinal locations with 50  $\mu$ l (total) of inoculum. Inoculated plants were examined daily and the appearance and severity of systemic symptoms recorded. Symptoms on any leaf above the inoculated leaf were considered to be systemic.

Typically, inoculation of Burley 49 plants with TEV (either purified virus or plant sap) resulted in severe chlorosis and mosaic and mottle on systemically infected leaves approximately 6-7 days after inoculation. Severe etching of the leaf followed within a few days. It was observed that transgenic plants containing only the CaMV promoter and untranslated sequences (i.e., 35S plant line) responded to challenge inoculation in a manner similar to wild type Burley 49, developing extensive chlorosis and etching at the same

rate (Fig. 4A). Plant lines which expressed FL TEV CP showed little or no delay in the appearance of symptoms when inoculated with infected plant sap. However, FL transgenic plants did show a slight attenuation of symptoms and eventually (2-4 weeks after initial appearance of symptoms), younger leaf tissue emerged devoid of symptoms and virus as demonstrated by back inoculation experiments. Typically chlorosis and etching on older systemic leaves was limited.

10 Ten independently transformed RC lines and seven independently transformed AS lines were obtained. Progeny from three of the RC lines, including line RC #5 and from one of the AS lines, including AS #3, showed an altered response to viral infection relative to control plants. All of these lines were verified to be transformed and were producing expected RNA products. A possible explanation for the variation in observed phenotype is the previously noted "position effect" whereby the expression of genes from identical DNA sequences integrated at different locations within the genome show varying patterns of tissue specificity.

20 Ten R2 expressing plants of the FL expressing line were inoculated with infected plant sap, and 20 R1 plants of lines AS #3 and RC #5 were inoculated with 50  $\mu$ l of a 5  $\mu$ g/ml solution of purified TEV. Identical results to those obtained by purified TEV inoculation were obtained when AS #3 and RC #5 R1 plants were inoculated with TEV-infected plant sap, as described above.

30 Transgenic Burley 49 plant lines AS #3 and RC #5, expressing only TEV CP related RNA sequences, showed a delay in the appearance of symptoms and a modification of symptoms when inoculated with TEV (Fig. 4B). Since the 20 R1 plants were not screened for expression of CP RNA prior to inoculation, some of the symptomatic plants represented non-expressing plants in which the gene of interest had been lost during Mendelian segregation. Modified symptoms on AS #3 plants appeared as small

chlorotic lesions often associated with a vein. Most of the leaves were devoid of symptoms and virus (determined by back inoculation experiments). Approximately 15% of RC #5 plants showed symptoms which were identical to those of infected Burley 49. However, the remaining RC #5 plants were entirely asymptomatic, and virus was not detected in back inoculation studies.

Plants from TEV resistant AS and RC lines showed no increased resistance, relative to untransformed controls, to infection by two other members of the potyvirus family, namely Tobacco Vein Mottling Virus and Potato Virus Y.

R<sub>2</sub> generation plants derived from TEV-resistant RC plants showed the expected Mendelian pattern of inheritance of the TEV-resistant phenotype.

Analysis of TEV Replication in Protoplasts Derived from Transgenic Plant Lines

In an attempt to explain the results obtained when AS and RC transgenic plants were challenged with TEV, it was sought to determine if all of the transgenic plant lines would support virus replication at a level comparable to Burley 49. Accumulation of viral encoded proteins was used as an indirect indicator of viral replication. Protoplasts were derived from leaf tissue of homozygous CP expressing plants and electroporated according to the procedure of Luciano et al. (1987) with TEV RNA. Protoplasts were prepared from transgenic plants and electroporated according to the procedure of Luciano et al. (1987). Protoplasts ( $1 \times 10^6$ ) were resuspended in 450  $\mu$ l electroporation buffer (330 mM mannitol, 1 mM KPO<sub>4</sub> pH 7.0, 150 mM KCl) and electroporated using a BTX Transfecto 300 (BTX San Diego, CA) (950 micro Farads, 130-volt pulse amplitude, 3.5 mm electrode gap) in the presence or absence of 6  $\mu$ g of purified TEV RNA. After electroporation, protoplasts were incubated for 96 hours in incubation medium as described in Luciano et al. (1987). Protoplasts were extracted in 2X Laemmli (Trisglycine) running buffer,

and  $5 \times 10^4$  extracted protoplasts were then subjected to western blot analysis as described above. Protoplast viability was measured by dye exclusion as described in Luciano et al. (1987). All electroporated protoplast samples had equivalent viability counts. The results indicated that protoplasts from all FL plant lines supported virus replication at levels comparable to wild type Burley 49 protoplasts. R1 transgenic plants from lines AS #3 and RC #5 were initially screened by northern analysis, and leaves from positive expressors were used in the production of protoplasts. Transfected protoplasts derived from AS #3 plants supported TEV replication, albeit at a reduced level. Protoplasts derived from RC #5 transgenic plant leaf tissue did not support TEV replication at a detectable level. These results, and those presented in the whole plant inoculation series, suggested AS and RC plants interfere with TEV replication.

#### Discussion of Data

The above example indicates that varying degrees of protection from TEV infection can be achieved by overexpression of coat protein and by expression of an antisense RNA. The current invention which comprises the expression of an untranslatable plus sense RNA molecule provides protection against TEV infection that is more effective than either of these two methods. Plants of line RC #5, transformed with the disclosed DNA molecule encoding an untranslatable plus sense RNA derived from the TEV coat protein gene, were asymptomatic and appear to be completely protected from virus infection. The disclosed invention therefore represents a new and effective way of generating potyvirus resistant germplasm.

Tobacco protoplasts derived from plants expressing the antisense RNA supported a reduced level of TEV replication compared to control cells derived from untransformed plants. In contrast, tobacco protoplasts derived from plants of line RC #5,

expressing the untranslatable plus sense RNA did not support detectable TEV replication. This suggests that the untranslatable plus sense RNA was more effective at blocking TEV replication in the cells of those transformed plants tested.

It is proposed that the untranslatable plus sense RNA inhibits viral replication by hybridizing to the minus sense RNA replicative template of TEV. The finding that plants expressing untranslatable plus sense RNA derived from the TEV coat protein gene are not protected from infection by Potato Virus Y or Tobacco Vein Mottling Virus is therefore explained by the circa 40-50% amino acid sequence divergence between the coat proteins of these viruses and TEV (Allison et al. 1986; Robaglia et al. 1989; Domier et al. 1986).

From the above-described findings, it would be reasonable and entirely predictable that if plants were transformed with a gene encoding an untranslatable plus sense RNA derived from a gene which was highly conserved between viruses of the potyvirus family, that these plants would be protected from infection by a wide range of viruses. Regions of the potyvirus genome which are sufficiently conserved between potyvirus types to be potentially useful in such an approach may be readily determined by one skilled in the art. Highly conserved regions may be determined by reference to published sequence data (Allison et al. 1986; Robaglia et al. 1989; Domier et al. 1986; Lain et al. 1989; Maiss et al. 1989). The utility of the identified regions could be readily determined using the methodologies described above and substituting the defined region for the TEV coat protein gene.

Regions of the potyvirus genome potentially suitable include, but are not limited to the genes encoding the viral replicase and the viral proteinase. Furthermore, it will be apparent to one skilled in the art that highly conserved portions of a particular gene may also serve in this role.

It will also be apparent to one skilled in the art that the described invention may also be used to produce plants resistant to viruses outside of the potyvirus family in instances where these viruses also produce a minus sense RNA replicative template.

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## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

- (i) APPLICANT: William G. Dougherty and  
John A. Lindbo
- 5 (ii) TITLE OF INVENTION: Production of Plants  
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Plus Sense RNA Molecules
- (iii) NUMBER OF SEQUENCES: 4
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- (v) COMPUTER READABLE FORM:
- (A) MEDIUM TYPE: Diskette, 5.25 inch
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- (2) INFORMATION FOR SEQ ID NO: 1:
- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 9495
- (B) TYPE: Nucleic Acid
- 40 (C) STRANDEDNESS: Single

-28-

(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE:

(A) DESCRIPTION: cDNA to genomic RNA

(iii) HYPOTHETICAL: No

5 (iv) ANTI-SENSE: No

(v) FRAGMENT TYPE: N/A

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Tobacco Etch Virus (TEV)

(B) STRAIN: Highly Aphid Transmitted (HAT)

10 (vii) IMMEDIATE SOURCE: TEV propagated in N. tabacum Burley 49

(viii) POSITION IN GENOME: N/A

(ix) FEATURE:

15 (A) NAME/KEY: Coat protein gene

(B) LOCATION: Genomic nucleotides 8518-9306

(C) IDENTIFICATION METHOD: --

20 (D) OTHER INFORMATION: SEQ. ID No. 1 is the cDNA corresponding to the Tobacco Etch Virus Genome.

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NAAATAACAA ATCTCAACAC AACATATACA AAACAAACGA ATCTCAAGCA ATCAAGCATT 60

CTACTTCTAT TGCAGCAATT TAAATCATTT CTTTTAAAGC AAAAGCAATT TTCTGAAAAT 120

5 TTTCACCATT TACGAACGAT AGCA ATG GCA CTG ATC TTT GGC ACA GTC AAC GCT 174

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1 5 10

10 AAC ATC CTG AAG GAA GTG TTC GGT GGA GCT CGT ATG GCT TGC GTT ACC 222

Asn Ile Leu Lys Glu Val Phe Gly Gly Ala Arg Met Ala Cys Val Thr

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	460 465 470	
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	475 480 485 490	
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	Ser Ser Lys Ala His Val Asn Asn Ala Leu Met Cys Asp Asn Gln Leu	
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	His Leu Val Ile Gly Asn Ser Gly Asp Ser Lys Tyr Leu Asp Leu Pro	
	620 625 630	
20	GTT CTC AAT GAA GAG AAA ATG TAT ATA GCT AAT GAA GGT TAT TGC TAC	2094
	Val Leu Asn Glu Glu Lys Met Tyr Ile Ala Asn Glu Gly Tyr Cys Tyr	
	635 640 645 650	
25	ATG AAC ATT TTC TTT GCT CTA CTA GTG AAT GTC AAG GAA GAG GAT GCA	2142
	Met Asn Ile Phe Phe Ala Leu Leu Val Asn Val Lys Glu Glu Asp Ala	
	655 660 665	
30	AAG GAC TTC ACC AAG TTT ATA AGG GAC ACA ATT GTT CCA AAG CTT GGA	2190
	Lys Asp Phe Thr Lys Phe Ile Arg Asp Thr Ile Val Pro Lys Leu Gly	
	670 675 680	
35	GCG TGG CCA ACA ATG CAA GAT GTT GCA ACT GCA TGC TAC TTA CTT TCC	2238
	Ala Trp Pro Thr Met Gln Asp Val Ala Thr Ala Cys Tyr Leu Leu Ser	
	685 690 695	
40	ATT CTT TAC CCA GAT GTC CTG AGA GCT GAA CTA CCC AGA ATT TTG GTT	2286
	Ile Leu Tyr Pro Asp Val Leu Arg Ala Glu Leu Pro Arg Ile Leu Val	
	700 705 710	
45	GAT CAT GAC AAC AAA ACA ATG CAT GTT TTG GAT TCG TAT GGG TCT AGA	2334
	Asp His Asp Asn Lys Thr Met His Val Leu Asp Ser Tyr Gly Ser Arg	
	715 720 725 730	
50	ACG ACA GGA TAC CAC ATG TTG AAA ATG AAC ACA ACA TCC CAG CTA ATT	2382
	Thr Thr Gly Tyr His Met Leu Lys Met Asn Thr Thr Ser Gln Leu Ile	
	735 740 745	
55	GAA TTC GTT CAT TCA GGT TTG GAA TCC GAA ATG AAA ACT TAC AAT GTT	2430
	Glu Phe Val His Ser Gly Leu Glu Ser Glu Met Lys Thr Tyr Asn Val	
	750 755 760	
60	GGA GGG ATG AAC CGA GAT GTG GTC ACA CAA GGT GCA ATT GAG ATG TTG	2478
	Gly Gly Met Asn Arg Asp Val Val Thr Gln Gly Ala Ile Glu Met Leu	
	765 770 775	
65	ATC AAG TCT ATA TAC AAA CCA CAT CTC ATG AAG CAG TTA CTT GAG GAA	2526
	Ile Lys Ser Ile Tyr Lys Pro His Leu Met Lys Gln Leu Leu Glu Glu	
	780 785 790	
70	GAG CCA TAC ATA ATT GTC CTG GCA ATA GTC TCC CCT TCA ATT TTA ATT	2574
	Glu Pro Tyr Ile Ile Val Leu Ala Ile Val Ser Pro Ser Ile Leu Ile	
	795 800 805 810	
75	GCC ATG TAC AAC TCT GGA ACT TTT GAG CAG GCG TTA CAA ATG TGG TTG	2622
	Ala Met Tyr Asn Ser Gly Thr Phe Glu Gln Ala Leu Gln Met Trp Leu	
	815 820 825	
80	CCA AAT ACA ATG AGG TTA GCT AAC CTC GCT GCC ATC TTG TCA GCC TTA	2670
	Pro Asn Thr Met Arg Leu Ala Asn Leu Ala Ala Ile Leu Ser Ala Leu	
	830 835 840	

	GCG CAA AAG TTA ACT TTG GCA GAT TTG TTC GTC CAG CAG CGT AAT TTG	2718
	Ala Gln Lys Leu Thr Leu Ala Asp Leu Phe Val Gln Gln Arg Asn Leu	
	845 850 855	
5	ATT AAT GAG TAT GCG CAG GTA ATT TTG GAC AAT CTG ATT GAC GGT GTC	2766
	Ile Asn Glu Tyr Ala Gln Val Ile Leu Asp Asn Leu Ile Asp Gly Val	
	860 865 870	
10	AGG GTT AAT CAT TCG CTA TCC CTA GCA ATG GAA ATT GTT ACT ATT AAG	2814
	Arg Val Asn His Ser Leu Ser Leu Ala Met Glu Ile Val Thr Ile Lys	
	875 880 885 890	
15	CTG GCC ACC CAA GAG ATG GAC ATG GCG TTG AGG GAA GGT GGC TAT GCT	2862
	Leu Ala Thr Gln Glu Met Asp Met Ala Leu Arg Glu Gly Gly Tyr Ala	
	895 900 905	
20	GTG ACC TCT GAA AAG GTG CAT GAA ATG TTG GAA AAA AAC TAT GTA AAG	2910
	Val Thr Ser Glu Lys Val His Glu Met Leu Glu Lys Asn Tyr Val Lys	
	910 915 920	
25	GCT TTG AAG GAT GCA TGG GAC GAA TTA ACT TGG TTG GAA AAA TTC TCC	2958
	Ala Leu Lys Asp Ala Trp Asp Glu Leu Thr Trp Leu Glu Lys Phe Ser	
	925 930 935	
30	GCA ATC AGG CAT TCA AGA AAG CTC TTG AAA TTT GGG CGA AAG CCT TTA	3006
	Ala Ile Arg His Ser Arg Lys Leu Leu Lys Phe Gly Arg Lys Pro Leu	
	940 945 950	
35	ATC ATG AAA AAC ACC GTA GAT TGC GGC GGA CAT ATA GAC TTG TCT GTG	3054
	Ile Met Lys Asn Thr Val Asp Cys Gly Gly His Ile Asp Leu Ser Val	
	955 960 965 970	
40	AAA TCG CTT TTC AAG TTC CAC TTG GAA CTC CTG AAG GGA ACC ATC TCA	3102
	Lys Ser Leu Phe Lys Phe His Leu Glu Leu Leu Lys Gly Thr Ile Ser	
	975 980 985	
45	AGA GCC GTA AAT GGT GGC GCA AGA AAG GTA AGA GTA GCG AAG AAT GCC	3150
	Arg Ala Val Asn Gly Gly Ala Arg Lys Val Arg Val Ala Lys Asn Ala	
	990 995 1000	
50	ATG ACA AAA GGG GTT TTT CTC AAA ATC TAC AGC ATG CTT CCT GAC GTC	3198
	Met Thr Lys Gly Val Phe Leu Lys Ile Tyr Ser Met Leu Pro Asp Val	
	1005 1010 1015	
55	TAC AAG TTT ATC ACA GTC TCG AGT GTC CTT TCC TTG TTG TTG ACA TTC	3246
	Tyr Lys Phe Ile Thr Val Ser Ser Val Leu Ser Leu Leu Leu Thr Phe	
	1020 1025 1030	
60	TTA TTT CAA ATT GAC TGC ATG ATA AGG GCA CAC CGA GAG GCG AAG GTT	3294
	Leu Phe Gln Ile Asp Cys Met Ile Arg Ala His Arg Glu Ala Lys Val	
	1035 1040 1045 1050	
65	GCT GCA CAG TTG CAG AAA GAG AGC GAG TGG GAC AAT ATC ATC AAT AGA	3342
	Ala Ala Gln Leu Gln Lys Glu Ser Glu Trp Asp Asn Ile Ile Asn Arg	
	1055 1060 1065	
70	ACT TTC CAG TAT TCT AAG CTT GAA AAT CCT ATT GGC TAT CGC TCT ACA	3390
	Thr Phe Gln Tyr Ser Lys Leu Glu Asn Pro Ile Gly Tyr Arg Ser Thr	
	1070 1075 1080	
75	GCG GAG GAA AGA CTC CAA TCA GAA CAC CCC GAG GCT TTC GAG TAC TAC	3438
	Ala Glu Glu Arg Leu Gln Ser Glu His Pro Glu Ala Phe Glu Tyr Tyr	
	1085 1090 1095	
80	AAG TTT TGC ATT GGA AAG GAA GAC CTC GTT GAA CAG GCA AAA CAA CCG	3486
	Lys Phe Cys Ile Gly Lys Glu Asp Leu Val Glu Gln Ala Lys Gln Pro	
	1100 1105 1110	

	GAG ATA GCA TAC TTT GAA AAG ATT ATA GCT TTC ATC ACA CTT GTA TTA	3534
	Glu Ile Ala Tyr Phe Glu Lys Ile Ile Ala Phe Ile Thr Leu Val Leu	
	1115 1120 1125 1130	
5	ATG GCT TTT GAC GCT GAG CCG AGT GAT GGA GTG TTC AAG ATA CTC AAT	3582
	Met Ala Phe Asp Ala Glu Arg Ser Asp Gly Val Phe Lys Ile Leu Asn	
	1135 1140 1145	
10	AAG TTC AAA GGA ATA CTG AGC TCA ACG GAG AGG GAG ATC ATC TAC ACG	3630
	Lys Phe Lys Gly Ile Leu Ser Ser Thr Glu Arg Glu Ile Ile Tyr Thr	
	1150 1155 1160	
15	CAG AGT TTG GAT GAT TAC GTT ACA ACC TTT GAT GAC AAT ATG ACA ATC	3678
	Gln Ser Leu Asp Asp Tyr Val Thr Thr Phe Asp Asp Asn Met Thr Ile	
	1165 1170 1175	
20	AAC CTC GAG TTG AAT ATG GAT GAA CTC CAC AAG ACG AGC CTT CCT GGA	3726
	Asn Leu Glu Leu Asn Met Asp Glu Leu His Lys Thr Ser Leu Pro Gly	
	1180 1185 1190	
25	GTC ACT TTT AAG CAA TGG TGG AAC AAC CAA ATC AGC CGA GGC AAC GTG	3774
	Val Thr Phe Lys Gln Trp Trp Asn Asn Gln Ile Ser Arg Gly Asn Val	
	1195 1200 1205 1210	
30	AAG CCA CAT TAT AGA ACT GAG GGG CAC TTC ATG GAG TTT ACC AGA GAT	3822
	Lys Pro His Tyr Arg Thr Glu Gly His Phe Met Glu Phe Thr Arg Asp	
	1215 1220 1225	
35	ACT GCG GCA TCG GTT GCC AGC GAG ATA TCA CAC TCA CCC GCA AGA GAT	3870
	Thr Ala Ala Ser Val Ala Ser Glu Ile Ser His Ser Pro Ala Arg Asp	
	1230 1235 1240	
40	TTT CTT GTG AGA GGT GCT GTT GGA TCT GGA AAA TCC ACA GGA CTT CCA	3918
	Phe Leu Val Arg Gly Ala Val Gly Ser Gly Lys Ser Thr Gly Leu Pro	
	1245 1250 1255	
45	TAC CAT TTA TCA AAG AGA GGG AGA GTG TTA ATG CTT GAG CCT ACC AGA	3966
	Tyr His Leu Ser Lys Arg Gly Arg Val Leu Met Leu Glu Pro Thr Arg	
	1260 1265 1270	
50	CCA CTC ACA GAT AAC ATG CAC AAG CAA CTG AGA AGT GAA CCA TTT AAC	4014
	Pro Leu Thr Asp Asn Met His Lys Gln Leu Arg Ser Glu Pro Phe Asn	
	1275 1280 1285 1290	
55	TGC TTC CCA ACT TTG AGG ATG AGA GGG AAG TCA ACT TTT GGG TCA TCA	4062
	Cys Phe Pro Thr Leu Arg Met Arg Gly Lys Ser Thr Phe Gly Ser Ser	
	1295 1300 1305	
60	CCG ATC ACA GTC ATG ACT AGT GGA TTC GCT TTA CAC CAC TTT GCA CGA	4110
	Pro Ile Thr Val Met Thr Ser Gly Phe Ala Leu His His Phe Ala Arg	
	1310 1315 1320	
65	AAC ATA GCT GAG GTA AAA ACA TAC GAT TTT GTC ATA ATT GAT GAA TGT	4158
	Asn Ile Ala Glu Val Lys Thr Tyr Asp Phe Val Ile Ile Asp Glu Cys	
	1325 1330 1335	
70	CAT GTG AAT GAT GCT TCT GCT ATA GCG TTT AGG AAT CTA CTG TTT GAA	4206
	His Val Asn Asp Ala Ser Ala Ile Ala Phe Arg Asn Leu Leu Phe Glu	
	1340 1345 1350	
75	CAT GAA TTT GAA GGA AAA GTC CTC AAA GTG TCA GCC ACA CCA CCA GGT	4254
	His Glu Phe Glu Gly Lys Val Leu Lys Val Ser Ala Thr Pro Pro Gly	
	1355 1360 1365 1370	
80	AGA GAA GTT GAA TTT ACA ACT CAG TTT CCC GTG AAA CTC AAG ATA GAA	4302
	Arg Glu Val Glu Phe Thr Thr Gln Phe Pro Val Lys Leu Lys Ile Glu	
	1375 1380 1385	

	GAG GCT CTT AGC TTT CAG GAA TTT GTA AGT TTA CAA GGG ACA GGT GCC	4350
	Glu Ala Leu Ser Phe Gln Glu Phe Val Ser Leu Gln Gly Thr Gly Ala	
	1390 1395 1400	
5	AAC GCC GAT GTG ATT AGT TGT GGC GAC AAC ATA CTA GTA TAT GTT GCT	4398
	Asn Ala Asp Val Ile Ser Cys Gly Asp Asn Ile Leu Val Tyr Val Ala	
	1405 1410 1415	
10	AGC TAC AAT GAT GTT GAT AGT CTT GGC AAG CTC CTT GTG CAA AAG GGA	4446
	Ser Tyr Asn Asp Val Asp Ser Leu Gly Lys Leu Leu Val Gln Lys Gly	
	1420 1425 1430	
15	TAC AAA GTG TCG AAG ATT GAT GGA AGA ACA ATG AAG AGT GGA GGA ACT	4494
	Tyr Lys Val Ser Lys Ile Asp Gly Arg Thr Met Lys Ser Gly Gly Thr	
	1435 1440 1445 1450	
20	GAA ATA ATC ACT GAA GGT ACT TCA GTG AAA AAG CAT TTC ATA GTC GCA	4542
	Glu Ile Ile Thr Glu Gly Thr Ser Val Lys Lys His Phe Ile Val Ala	
	1455 1460 1465	
25	ACT AAC ATT ATT GAG AAT GGT GTA ACC ATT GAC ATT GAT GTA GTT GTG	4590
	Thr Asn Ile Ile Glu Asn Gly Val Thr Ile Asp Ile Asp Val Val Val	
	1470 1475 1480	
30	GAT TTT GGG ACT AAG GTT GTA CCA GTT TTG GAT GTG GAC AAT AGA GCG	4638
	Asp Phe Gly Thr Lys Val Val Pro Val Leu Asp Val Asp Asn Arg Ala	
	1481 1490 1495	
35	GTG CAG TAC AAC AAA ACT GTG GTG AGT TAT GGG GAG CGC ATC CAA AAA	4686
	Val Gln Tyr Asn Lys Thr Val Val Ser Tyr Gly Glu Arg Ile Gln Lys	
	1500 1505 1510	
40	CTC GGT AGA GTT GGG CGA CAC AAG GAA GGA GTA GCA CTT CGA ATT GGC	4734
	Leu Gly Arg Val Gly Arg His Lys Glu Gly Val Ala Leu Arg Ile Gly	
	1515 1520 1525 1530	
45	CAA ACA AAT AAA ACA CTG GTT GAA ATT CCA GAA ATG GTT GCC ACT GAA	4782
	Gln Thr Asn Lys Thr Leu Val Glu Ile Pro Glu Met Val Ala Thr Glu	
	1535 1540 1545	
50	GCT GCC TTT CTA TGC TTC ATG TAC AAT TTG CCA GTG ACA ACA CAG AGT	4830
	Ala Ala Phe Leu Cys Phe Met Tyr Asn Leu Pro Val Thr Thr Gln Ser	
	1550 1555 1560	
55	GTT TCA ACC ACA CTG CTG GAA AAT GCC ACA TTA TTA CAA GCT AGA ACT	4878
	Val Ser Thr Thr Leu Leu Glu Asn Ala Thr Leu Leu Gln Ala Arg Thr	
	1565 1570 1575	
60	ATG GCA CAG TTT GAG CTA TCA TAT TTT TAC ACA ATT AAT TTT GTG CGA	4926
	Met Ala Gln Phe Glu Leu Ser Tyr Phe Tyr Thr Ile Asn Phe Val Arg	
	1580 1585 1590	
65	TTT GAT GGT AGT ATG CAT CCA GTC ATA CAT GAC AAG CTG AAG CGC TTT	4974
	Phe Asp Gly Ser Met His Pro Val Ile His Asp Lys Leu Lys Arg Phe	
	1595 1600 1605 1610	
70	AAG CTA CAC ACT TGT GAG ACA TTC CTC AAT AAG TTG GCG ATC CCA AAT	5022
	Lys Leu His Thr Cys Glu Thr Phe Leu Asn Lys Leu Ala Ile Pro Asn	
	1615 1620 1625	
75	AAA GGC TTA TCC TCT TGG CTT ACG AGT GGA GAG TAT AAG CGA CTT GGT	5070
	Lys Gly Leu Ser Ser Trp Leu Thr Ser Gly Glu Tyr Lys Arg Leu Gly	
	1630 1635 1640	
80	TAC ATA GCA GAG GAT GCT GGC ATA AGA ATC CCA TTC GTG TGC AAA GAA	5118
	Tyr Ile Ala Glu Asp Ala Gly Ile Arg Ile Pro Phe Val Cys Lys Glu	
	1645 1650 1655	

	ATT CCA GAC TCC TTG CAT GAG GAA ATT TGG CAC ATT GTA GTC GCC CAT	5166
	Ile Pro Asp Ser Leu His Glu Glu Ile Trp His Ile Val Val Ala His	
	1660 1665 1670	
5	AAA GGT GAC TCG GGT ATT GGG AGG CTC ACT AGC GTA CAG GCA GCA AAG	5214
	Lys Gly Asp Ser Gly Ile Gly Arg Leu Thr Ser Val Gln Ala Ala Lys	
	1675 1680 1685 1690	
10	GTT GTT TAT ACT CTG CAA ACG GAT GTG CAC TCA ATT GCG AGG ACT CTA	5262
	Val Val Tyr Thr Leu Gln Thr Asp Val His Ser Ile Ala Arg Thr Leu	
	1695 1700 1705	
15	GCA TGC ATC AAT AGA CGC ATA GCA GAT GAA CAA ATG AAG CAG AGT CAT	5310
	Ala Cys Ile Asn Arg Arg Ile Ala Asp Glu Gln Met Lys Gln Ser His	
	1710 1715 1720	
20	TTT GAA GCC GCA ACT GGG AGA GCA TTT TCC TTC ACA AAT TAC TCA ATA	5358
	Phe Glu Ala Ala Thr Gly Arg Ala Phe Ser Phe Thr Asn Tyr Ser Ile	
	1725 1730 1735	
25	CAA AGC ATA TTT GAC ACG CTG AAA GCA AAT TAT GCT ACA AAG CAT ACG	5406
	Gln Ser Ile Phe Asp Thr Leu Lys Ala Asn Tyr Ala Thr Lys His Thr	
	1740 1745 1750	
30	AAA GAA AAT ATT GCA GTG CTT CAG CAG GCA AAA GAT CAA TTG CTA GAG	5454
	Lys Glu Asn Ile Ala Val Leu Gln Gln Ala Lys Asp Gln Leu Leu Glu	
	1755 1760 1765 1770	
35	TTT TCG AAC CTA GCA AAG GAT CAA GAT GTC ACG GGT ATC ATC CAA GAC	5502
	Phe Ser Asn Leu Ala Lys Asp Gln Asp Val Thr Gly Ile Ile Gln Asp	
	1775 1780 1785	
40	TTC AAT CAC CTG GAA ACT ATC TAT CTC CAA TCA GAT AGC GAA GTG GCT	5550
	Phe Asn His Leu Glu Thr Ile Tyr Leu Gln Ser Asp Ser Glu Val Ala	
	1790 1795 1800	
45	AAG CAT CTG AAG CTT AAA AGT CAC TGG AAT AAA AGC CAA ATC ACT AGG	5598
	Lys His Leu Lys Leu Lys Ser His Trp Asn Lys Ser Gln Ile Thr Arg	
	1805 1810 1815	
50	GAC ATC ATA ATA GCT TTG TCT GTG TTA ATT GGT GGT GGA TGG ATG CTT	5646
	Asp Ile Ile Ile Ala Leu Ser Val Leu Ile Gly Gly Gly Trp Met Leu	
	1820 1825 1830	
55	GCA ACG TAC TTC AAG GAC AAG TTC AAT GAA CCA GTC TAT TTC CAA GGG	5694
	Ala Thr Tyr Phe Lys Asp Lys Phe Asn Glu Pro Val Tyr Phe Gln Gly	
	1835 1840 1845 1850	
60	AAG AAG AAT CAG AAG CAC AAG CTT AAG ATG AGA GAG GCG CGT GGG GCT	5742
	Lys Lys Asn Gln Lys His Lys Leu Lys Met Arg Glu Ala Arg Gly Ala	
	1855 1860 1865	
65	AGA GGG CAA TAT GAG GTT GCA GCG GAG CCA GAG GCG CTA GAA CAT TAC	5790
	Arg Gly Gln Tyr Glu Val Ala Ala Glu Pro Glu Ala Leu Glu His Tyr	
	1870 1875 1880	
70	TTT GGA AGC GCA TAT AAT AAC AAA GGA AAG CGC AAG GGC ACC ACG AGA	5838
	Phe Gly Ser Ala Tyr Asn Asn Lys Gly Lys Arg Lys Gly Thr Thr Arg	
	1885 1890 1895	
75	GGA ATG GGT GCA AAG TCT CGG AAA TTC ATA AAC ATG TAT GGG TTT GAT	5886
	Gly Met Gly Ala Lys Ser Arg Lys Phe Ile Asn Met Tyr Gly Phe Asp	
	1900 1905 1910	
80	CCA ACT GAT TTT TCA TAC ATT AGG TTT GTG GAT CCA TTG ACA GGT CAC	5934
	Pro Thr Asp Phe Ser Tyr Ile Arg Phe Val Asp Pro Leu Thr Gly His	
	1915 1920 1925 1930	

	ACT ATT GAT GAG TCC ACA AAC GCA CCT ATT GAT TTA GTG CAG CAT GAG	5982
	Thr Ile Asp Glu Ser Thr Asn Ala Pro Ile Asp Leu Val Gln His Glu	
	1935 1940 1945	
5	TTT GGA AAG GTT AGA ACA CGC ATG TTA ATT GAC GAT GAG ATA GAG CCT	6030
	Phe Gly Lys Val Arg Thr Arg Met Leu Ile Asp Asp Glu Ile Glu Pro	
	1950 1955 1960	
10	CAA AGT CTT AGC ACC CAC ACC ACA ATC CAT GCT TAT TTG GTG AAT AGT	6078
	Gln Ser Leu Ser Thr His Thr Thr Ile His Ala Tyr Leu Val Asn Ser	
	1965 1970 1975	
15	GGC ACG AAG AAA GTT CTT AAG GTT GAT TTA ACA CCA CAC TCG TCG CTA	6126
	Gly Thr Lys Lys Val Leu Lys Val Asp Leu Thr Pro His Ser Ser Leu	
	1980 1985 1990	
20	CGT GCG AGT GAG AAA TCA ACA GCA ATA ATG GGA TTT CCT GAA AGG GAG	6174
	Arg Ala Ser Glu Lys Ser Thr Ala Ile Met Gly Phe Pro Glu Arg Glu	
	1995 2000 2005 2010	
25	AAT GAA TTG CGT CAA ACC GGC ATG GCA GTG CCA GTG GCT TAT GAT CAA	6222
	Asn Glu Leu Arg Gln Thr Gly Met Ala Val Pro Val Ala Tyr Asp Gln	
	2015 2020 2025	
30	TTG CCA CCA AAG AAT GAG GAC TTG ACG TTT GAA GGA GAA AGC TTG TTT	6270
	Leu Pro Pro Lys Asn Glu Asp Leu Thr Phe Glu Gly Glu Ser Leu Phe	
	2030 2035 2040	
35	AAG GGA CCA CGT GAT TAC AAC CCG ATA TCG AGC ACC ATT TGT CAT TTG	6318
	Lys Gly Pro Arg Asp Tyr Asn Pro Ile Ser Ser Thr Ile Cys His Leu	
	2045 2050 2055	
40	ACG AAT GAA TCT GAT GGG CAC ACA ACA TCG TTG TAT GGT ATT GGA TTT	6366
	Thr Asn Glu Ser Asp Gly His Thr Thr Ser Leu Tyr Gly Ile Gly Phe	
	2060 2065 2070	
45	GGT CCC TTC ATC ATT ACA AAC AAG CAC TTG TTT AGA AGA AAT AAT GGA	6414
	Gly Pro Phe Ile Ile Thr Asn Lys His Leu Phe Arg Arg Asn Asn Gly	
	2075 2080 2085 2090	
50	ACA CTG TTG GTC CAA TCA CTA CAT GGT GTA TTC AAG GTC AAG AAC ACC	6462
	Thr Leu Leu Val Gln Ser Leu His Gly Val Phe Lys Val Lys Asn Thr	
	2095 2100 2105	
55	ACG ACT TTG CAA CAA CAC CTC ATT GAT GGG AGG GAC ATG ATA ATT ATT	6510
	Thr Thr Leu Gln His Leu Ile Asp Gly Arg Asp Met Ile Ile Ile	
	2110 2115 2120	
60	CGC ATG CCT AAG GAT TTC CCA CCA TTT CCT CAA AAG CTG AAA TTT AGA	6558
	Arg Met Pro Lys Asp Phe Pro Pro Phe Pro Gln Lys Leu Lys Phe Arg	
	2125 2130 2135	
65	GAG CCA CAA AGG GAA GAG CGC ATA TGT CTT GTG ACA ACC AAC TTC CAA	6606
	Glu Pro Gln Arg Glu Glu Arg Ile Cys Leu Val Thr Thr Asn Phe Gln	
	2140 2145 2150	
70	ACT AAG AGC ATG TCT AGC ATG GTG TCA GAC ACT AGT TGC ACA TTC CCT	6654
	Thr Lys Ser Met Ser Ser Met Val Ser Asp Thr Ser Cys Thr Phe Pro	
	2155 2160 2165 2170	
75	TCA TCT GAT GGC ATA TTC TGG AAG CAT TGG ATT CAA ACC AAG GAT GGG	6702
	Ser Ser Asp Gly Ile Phe Trp Lys His Trp Ile Gln Thr Lys Asp Gly	
	2175 2180 2185	
80	CAG TGT GGC AGT CCA TTA GTA TCA ACT AGA GAT GGG TTC ATT GTT GGT	6750
	Gln Cys Gly Ser Pro Leu Val Ser Thr Arg Asp Gly Phe Ile Val Gly	
	2190 2195 2200	

	ATA CAC TCA GCA TCG AAT TTC ACC AAC ACA AAC AAT TAT TTC ACA AGC Ile His Ser Ala Ser Asn Phe Thr Asn Thr Asn Asn Tyr Phe Thr Ser 2205 2210 2215	6798
5	GTG CCG AAA AAC TTC ATG GAA TTG TTG ACA AAT CAG GAG GCG CAG CAG Val Pro Lys Asn Phe Met Glu Leu Leu Thr Asn Gln Glu Ala Gln Gln 2220 2225 2230	6846
10	TGG GTT AGT GGT TGG CGA TTA AAT GCT GAC TCA GTA TTG TGG GGG GGC Trp Val Ser Gly Trp Arg Leu Asn Ala Asp Ser Val Leu Trp Gly Gly 2235 2240 2245 2250	6894
15	CAT AAA GTT TTC ATG AGC AAA CCT GAA GAG CCT TTT CAG CCA GTT AAG His Lys Val Phe Met Ser Lys Pro Glu Glu Pro Phe Gln Pro Val Lys 2255 2260 2265	6942
20	GAA GCG ACT CAA CTC ATG AAT GAA TTG GTG TAC TCG CAA GGG GAG AAG Glu Ala Thr Gln Leu Met Asn Glu Leu Val Tyr Ser Gln Gly Glu Lys 2270 2275 2280	6990
	AGG AAA TGG GTC GTG GAA GCA CTC TCA GGG AAC TTG AGG CCA GTG GCT Arg Lys Trp Val Val Glu Ala Leu Ser Gly Asn Leu Arg Pro Val Ala 2285 2290 2295	7038
25	GAG TGT CCC AGT CAG TTA GTC ACA AAG CAT GTG GTT AAA GGA AAG TGT Glu Cys Pro Ser Gln Leu Val Thr Lys His Val Val Lys Gly Lys Cys 2300 2305 2310	7086
30	CCC CTC TTT GAG CTC TAC TTG CAG TTG AAT CCA GAA AAG GAA GCA TAT Pro Leu Phe Glu Leu Tyr Leu Gln Leu Asn Pro Glu Lys Glu Ala Tyr 2315 2320 2325 2330	7134
35	TTT AAA CCG ATG ATG GGA GCA TAT AAG CCA AGT CGA CTT AAT AGA GAG Phe Lys Pro Met Met Gly Ala Tyr Lys Pro Ser Arg Leu Asn Arg Glu 2335 2340 2345	7182
40	GCG TTC CTC AAG GAC ATT CTA AAA TAT GCT AGT GAA ATT GAG ATT GGG Ala Phe Leu Lys Asp Ile Leu Lys Tyr Ala Ser Glu Ile Glu Ile Gly 2350 2355 2360	7230
	AAT GTG GAT TGT GAC TTG CTG GAG CTT GCA ATA AGC ATG CTC GTC ACA Asn Val Asp Cys Asp Leu Leu Glu Leu Ala Ile Ser Met Leu Val Thr 2365 2370 2375	7278
45	AAG CTC AAG GCG TTA GGA TTC CCA ACT GTG AAC TAC ATC ACT GAC CCA Lys Leu Lys Ala Leu Gly Phe Pro Thr Val Asn Tyr Ile Thr Asp Pro 2380 2385 2390	7326
50	GAG GAA ATT TTT AGT GCA TTG AAT ATG AAA GCA GCT ATG GGA GCA CTA Glu Glu Ile Phe Ser Ala Leu Asn Met Lys Ala Ala Met Gly Ala Leu 2395 2400 2405 2410	7374
55	TAC AAA GGC AAG AAG AAA GAA GCT CTC AGC GAG CTC ACA CTA GAT GAG Tyr Lys Gly Lys Lys Lys Glu Ala Leu Ser Glu Leu Thr Leu Asp Glu 2415 2420 2425	7422
60	CAG GAG GCA ATG CTC AAA GCA AGT TGC CTG CGA CTG TAT ACG GGA AAG Gln Glu Ala Met Leu Lys Ala Ser Cys Leu Arg Leu Tyr Thr Gly Lys 2430 2435 2440	7470
	TTG GGA ATT TGG AAT GGC TCA TTG AAA GCA GAG TTG CGT CCA ATT GAG Leu Gly Ile Trp Asn Gly Ser Leu Lys Ala Glu Leu Arg Pro Ile Glu 2445 2450 2455	7518
65	AAG GTT GAA AAC AAC AAA ACG CGA ACT TTC ACA GCA GCA CCA ATA GAC Lys Val Glu Asn Asn Lys Thr Arg Thr Phe Thr Ala Ala Pro Ile Asp 2460 2465 2470	7566

	ACT CTT CTT GCT GGT AAA GTT TGC GTG GAT GAT TTC AAC AAT CAA TTT Thr Leu Leu Ala Gly Lys Val Cys Val Asp Asp Phe Asn Asn Gln Phe 2475 2480 2485 2490	7614
5	TAT GAT CTC AAC ATA AAG GCA CCA TGG ACA GTT GGT ATG ACT AAG TTT Tyr Asp Leu Asn Ile Lys Ala Pro Trp Thr Val Gly Met Thr Lys Phe 2495 2500 2505	7662
10	TAT CAG GGG TGG AAT GAA TTG ATG GAG GCT TTA CCA AGT GGG TGG GTG Tyr Gln Gly Trp Asn Glu Leu Met Glu Ala Leu Pro Ser Gly Trp Val 2510 2515 2520	7710
15	TAT TGT GAC GCT GAT GGT TCG CAA TTC GAC AGT TCC TTG ACT CCA TTC Tyr Cys Asp Ala Asp Gly Ser Gln Phe Asp Ser Ser Leu Thr Pro Phe 2525 2530 2535	7758
20	CTC ATT AAT GCT GTA TTG AAA GTG CGA CTT GCC TTC ATG GAG GAA TGG Leu Ile Asn Ala Val Leu Lys Val Arg Leu Ala Phe Met Glu Glu Trp 2540 2545 2550	7806
	GAT ATT GGT GAG CAA ATG CTG CGA AAT TTG TAC ACT GAG ATA GTG TAT Asp Ile Gly Glu Gln Met Leu Arg Asn Leu Tyr Thr Glu Ile Val Tyr 2555 2560 2565 2570	7854
25	ACA CCA ATC CTC ACA CCG GAT GGT ACT ATC ATT AAG AAG CAT AAA GGC Thr Pro Ile Leu Thr Pro Asp Gly Thr Ile Ile Lys Lys His Lys Gly 2575 2580 2585	7902
30	AAC AAT AGC GGG CAA CCT TCA ACA GTG GTG GAC AAC ACA CTC ATG GTC Asn Asn Ser Gly Gln Pro Ser Thr Val Val Asp Asn Thr Leu Met Val 2590 2595 2600	7950
35	ATT ATT GCA ATG TTA TAC ACA TGT GAG AAG TGT GGA ATC AAC AAG GAA Ile Ile Ala Met Leu Tyr Thr Cys Glu Lys Cys Gly Ile Asn Lys Glu 2605 2610 2615	7998
40	GAG ATT GTG TAT TAC GTC AAT GGC GAT GAC CTA TTG ATT GCC ATT CAC Glu Ile Val Tyr Tyr Val Asn Gly Asp Asp Leu Leu Ile Ala Ile His 2620 2625 2630	8046
	CCA GAT AAA GCT GAG AGG TTG AGT AGA TTC AAA GAA TCT TTC GGA GAG Pro Asp Lys Ala Glu Arg Leu Ser Arg Phe Lys Glu Ser Phe Gly Glu 2635 2640 2645 2650	8094
45	TTG GGC CTG AAA TAT GAA TTT GAC TGT ACC ACC AGG GAC AAG ACA CAG Leu Gly Leu Lys Tyr Glu Phe Asp Cys Thr Thr Arg Asp Lys Thr Gln 2655 2660 2665	8142
50	TTG TGG TTC ATG TCA CAC AGG GCT TTG GAG AGG GAT GGC ATG TAT ATA Leu Trp Phe Met Ser His Arg Ala Leu Glu Arg Asp Gly Met Tyr Ile 2670 2675 2680	8190
55	CCA AAG CTA GAA GAA GAA AGG ATT GTT TCT ATT TTG GAA TGG GAC AGA Pro Lys Leu Glu Glu Glu Arg Ile Val Ser Ile Leu Glu Trp Asp Arg 2685 2690 2695	8238
60	TCC AAA GAG CCG TCA CAT AGG CTT GAA GCC ATC TGT GCA TCA ATG ATT Ser Lys Glu Pro Ser His Arg Leu Glu Ala Ile Cys Ala Ser Met Ile 2700 2705 2710	8286
	GAA GCA TGG GGT TAT GAC AAG CTG GTT GAA GAA ATC CGC AAT TTC TAT Glu Ala Trp Gly Tyr Asp Lys Leu Val Glu Glu Ile Arg Asn Phe Tyr 2715 2720 2725 2730	8334
65	GCA TGG GTT TTG GAA CAA GCG CCG TAT TCA CAG CTT GCA GAA GAA GGA Ala Trp Val Leu Glu Gln Ala Pro Tyr Ser Gln Leu Ala Glu Glu Gly 2735 2740 2745	8382

	AAG GCG CCA TAT CTG GCT GAG ACT GCG CTT AAG TTT TTG TAC ACA TCT	8430
	Lys Ala Pro Tyr Leu Ala Glu Thr Ala Leu Lys Phe Leu Tyr Thr Ser	
	2750 2755 2760	
5	CAG CAC GGA ACA AAC TCT GAG ATA GAA GAG TAT TTA AAA GTG TTG TAT	8478
	Gln His Gly Thr Asn Ser Glu Ile Glu Glu Tyr Leu Lys Val Leu Tyr	
	2765 2770 2775	
10	GAT TAC GAT ATT CCA ACG ACT GAG AAT CTT TAT TTT CAG AGT GGC ACT	8526
	Asp Tyr Asp Ile Pro Thr Thr Glu Asn Leu Tyr Phe Gln Ser Gly Thr	
	2780 2785 2790	
15	GTG GAT GCT GGT GCT GAC GCT GGT AAG AAG AAA GAT CAA AAG GAT GAT	8574
	Val Asp Ala Gly Ala Asp Ala Gly Lys Lys Lys Asp Gln Lys Asp Asp	
	2795 2800 2805 2810	
	AAA GTC GCT GAG CAG GCT TCA AAG GAT AGG GAT GTT AAT GCT GGA ACT	8622
	Lys Val Ala Glu Gln Ala Ser Lys Asp Arg Asp Val Asn Ala Gly Thr	
	2815 2820 2825	
20	TCA GGA ACA TTC TCA GTT CCA CGA ATA AAT GCT ATG GCC ACA AAA CTT	8670
	Ser Gly Thr Phe Ser Val Pro Arg Ile Asn Ala Met Ala Thr Lys Leu	
	2830 2835 2840	
25	CAA TAT CCA AGG ATG AGG GGA GAG GTG GTT GTA AAC TTG AAT CAC CTT	8718
	Gln Tyr Pro Arg Met Arg Gly Glu Val Val Val Asn Leu Asn His Leu	
	2845 2850 2855	
30	TTA GGA TAC AAG CCA CAG CAA ATT GAT TTG TCA AAT GCT CGA GCC ACA	8766
	Leu Gly Tyr Lys Pro Gln Ile Asp Leu Ser Asn Ala Arg Ala Thr	
	2860 2865 2870	
35	CAT GAG CAG TTT GCC GCG TGG CAT CAG GCA GTG ATG ACA GCC TAT GGA	8814
	His Glu Gln Phe Ala Ala Trp His Gln Ala Val Met Thr Ala Tyr Gly	
	2875 2880 2885 2890	
	GTG AAT GAA GAG CAA ATG AAA ATA TTG CTA AAT GGA TTT ATG GTG TGG	8862
	Val Asn Glu Glu Gln Met Lys Ile Leu Leu Asn Gly Phe Met Val Trp	
	2895 2900 2905	
40	TGC ATA GAA AAT GGG ACT TCC CCA AAT TTG AAC GGA ACT TGG GTT ATG	8910
	Cys Ile Glu Asn Gly Thr Ser Pro Asn Leu Asn Gly Thr Trp Val Met	
	2910 2915 2920	
45	ATG GAT GGT GAG GAT CAA GTT TCA TAC CCG CTG AAA CCA ATG GTT GAA	8958
	Met Asp Gly Glu Asp Gln Val Ser Tyr Pro Leu Lys Pro Met Val Glu	
	2925 2930 2935	
50	AAC GCG CAG CCA ACA CTG AGG CAA ATT ATG ACA CAC TTC AGT GAC CTG	9006
	Asn Ala Gln Pro Thr Leu Arg Gln Ile Met Thr His Phe Ser Asp Leu	
	2940 2945 2950	
55	GCT GAA GCG TAT ATT GAG ATG AGG AAT AGG GAG CGA CCA TAC ATG CCT	9054
	Ala Glu Ala Tyr Ile Glu Met Arg Asn Arg Glu Arg Pro Tyr Met Pro	
	2955 2960 2965 2970	
	AGG TAT GGT CTA CAG AGA AAC ATT ACA GAC ATG AGT TTG TCA CGC TAT	9102
	Arg Tyr Gly Leu Gln Arg Asn Ile Thr Asp Met Ser Leu Ser Arg Tyr	
	2975 2980 2985	
60	GCG TTC GAC TTC TAT GAG CTA ACT TCA AAA ACA CCT GTT AGA GCG AGG	9150
	Ala Phe Asp Phe Tyr Glu Leu Thr Ser Lys Thr Pro Val Arg Ala Arg	
	2990 2995 3000	
65	GAG GCG CAT ATG CAA ATG AAA GCT GCT GCA GTA CGA AAC AGT GGA ACT	9198
	Glu Ala His Met Gln Met Lys Ala Ala Ala Val Arg Asn Ser Gly Thr	
	3005 3010 3015	

	AGG TTA TTT GGT CTT GAT GGC AAC GTG GGT ACT GCA GAG GAA GAC ACT	9246
	Arg Leu Phe Gly Leu Asp Gly Asn Val Gly Thr Ala Glu Glu Asp Thr	
	3020 3025 3030	
5	GAA CGG CAC ACA GCG CAC GAT GTG AAC CGT AAC ATG CAC ACA CTA TTA	9294
	Glu Arg His Thr Ala His Asp Val Asn Arg Asn Met His Thr Leu Leu	
	3035 3040 3045 3050	
10	GGG GTC CGC CAG TGA TAGTTTCTGC GTGTCTTTGC TTTCCGCTTT TAAGCTTATT	9349
	Gly Val Arg Gln	
	GTAATATATA TGAATAGCTA TTCACAGTGG GACTTGGTCT TGTGTTGAAT AGTATCTTAT	9409
	ATATTTTAAT ATGTCTTATT AGTCTCATT CTTAGGCGAA CGACAAAGTG AGGTCACCTC	9469
15	GGTCTAATTC TCCTATGTAG TGCGAG	9495

## (3) INFORMATION FOR SEQ ID NO: 2:

- (i) SEQUENCE CHARACTERISTICS:
- 20 (A) LENGTH: 792
- (B) TYPE: Nucleic Acid
- (C) STRANDEDNESS: Double
- (D) TOPOLOGY: Circular
- (ii) MOLECULE TYPE: cDNA to genomic RNA
- 25 (iii) HYPOTHETICAL: No
- (iv) ANTI-SENSE: No
- (v) FRAGMENT TYPE: N/A
- (vi) ORIGINAL SOURCE:
- (A) ORGANISM: Tobacco Etch Virus
- 30 (B) STRAIN: Highly Aphid Transmitted
- (C) INDIVIDUAL ISOLATE: N/A
- (vii) IMMEDIATE SOURCE:
- (A) LIBRARY: No
- (B) CLONE: pTC:FL
- 35 (viii) POSITION IN GENOME: N/A
- (ix) FEATURE:
- (A) NAME/KEY: Mutations (AGT→ATG) introduced into nucleotides corresponding to genomic nucleotides 8518-8520 of SEQ ID No. 1, to create initiating methionine codon.
- 40 (B) LOCATION: Nucleotides 1-3 of SEQ ID No. 2
- (C) IDENTIFICATION METHOD: --
- 45 (D) OTHER INFORMATION: SEQ ID NO: 2 is the modified Tobacco Etch Virus coat protein gene present in pTC:FL.

## (x) PUBLICATION INFORMATION:

(A) AUTHORS: Allison et al.  
 (B) TITLE: The nucleotide sequence of the coding region of Tobacco Etch Virus Genomic RNA: Evidence for the Synthesis of a Single Polyprotein  
 (C) JOURNAL: Virology  
 (D) VOLUME: 154  
 (E) ISSUE: --  
 (F) PAGES: 9-20

(A) AUTHORS: Lindbo and Dougherty  
 (B) TITLE: Untranslatable Transcripts of the tobacco etch virus coat protein gene sequence can interfere with tobacco etch virus replication in Transgenic Plants and Protoplasts  
 (C) JOURNAL: Virology  
 (D) VOLUME: 189  
 (E) ISSUE: --  
 (F) PAGES: 725-733

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

		ATG GGC ACT	9
		Met Gly Thr	
25		1	
	GTG GAT GCT GGT GCT GAC GCT GGT AAG AAG AAA GAT CAA AAG GAT GAT	57	
	Val Asp Ala Gly Ala Asp Ala Gly Lys Lys Lys Asp Gln Lys Asp Asp		
	5 10 15		
30	AAA GTC GCT GAG CAG GCT TCA AAG GAT AGG GAT GTT AAT GCT GGA ACT	105	
	Lys Val Ala Glu Gln Ala Ser Lys Asp Arg Asp Val Asn Ala Gly Thr		
	20 25 30 35		
35	TCA GGA ACA TTC TCA GTT CCA CGA ATA AAT GCT ATG GCC ACA AAA CTT	153	
	Ser Gly Thr Phe Ser Val Pro Arg Ile Asn Ala Met Ala Thr Lys Leu		
	40 45 50		
40	CAA TAT CCA AGG ATG AGG GGA GAG GTG GTT GTA AAC TTG AAT CAC CTT	201	
	Gln Tyr Pro Arg Met Arg Gly Glu Val Val Asn Leu Asn His Leu		
	55 60 65		
45	TTA GGA TAC AAG CCA CAG CAA ATT GAT TTG TCA AAT GCT CGA GCC ACA	249	
	Leu Gly Tyr Lys Pro Gln Gln Ile Asp Leu Ser Asn Ala Arg Ala Thr		
	70 75 80		
50	CAT GAG CAG TTT GCC GCG TGG CAT CAG GCA GTG ATG ACA GCC TAT GGA	297	
	His Glu Gln Phe Ala Ala Trp His Gln Ala Val Met Thr Ala Tyr Gly		
	85 90 95		
55	GTG AAT GAA GAG CAA ATG AAA ATA TTG CTA AAT GGA TTT ATG GTG TGG	345	
	Val Asn Glu Glu Gln Met Lys Ile Leu Leu Asn Gly Phe Met Val Trp		
	100 105 110 115		
55	TGC ATA GAA AAT GGG ACT TCC CCA AAT TTG AAC GGA ACT TGG GTT ATG	393	
	Cys Ile Glu Asn Gly Thr Ser Pro Asn Leu Asn Gly Thr Trp Val Met		
	120 125 130		

	ATG GAT GGT GAG GAT CAA GTT TCA TAC CCG CTG AAA CCA ATG GTT GAA	441
	Met Asp Gly Glu Asp Gln Val Ser Tyr Pro Leu Lys Pro Met Val Glu	
	135 140 145	
5	AAC GCG CAG CCA ACA CTG AGG CAA ATT ATG ACA CAC TTC AGT GAC CTG	489
	Asn Ala Gln Pro Thr Leu Arg Gln Ile Met Thr His Phe Ser Asp Leu	
	150 155 160	
10	GCT GAA GCG TAT ATT GAG ATG AGG AAT AGG GAG CGA CCA TAC ATG CCT	537
	Ala Glu Ala Tyr Ile Glu Met Arg Asn Arg Glu Arg Pro Tyr Met Pro	
	165 170 175	
15	AGG TAT GGT CTA CAG AGA AAC ATT ACA GAC ATG AGT TTG TCA CGC TAT	585
	Arg Tyr Gly Leu Gln Arg Asn Ile Thr Asp Met Ser Leu Ser Arg Tyr	
	180 185 190 195	
20	GCG TTC GAC TTC TAT GAG CTA ACT TCA AAA ACA CCT GTT AGA GCG AGG	633
	Ala Phe Asp Phe Tyr Glu Leu Thr Ser Lys Thr Pro Val Arg Ala Arg	
	200 205 210	
25	GAG GCG CAT ATG CAA ATG AAA GCT GCT GCA GTA CGA AAC AGT GGA ACT	681
	Glu Ala His Met Gln Met Lys Ala Ala Val Arg Asn Ser Gly Thr	
	215 220 225	
30	AGG TTA TTT GGT CTT GAT GGC AAC GTG GGT ACT GCA GAG GAA GAC ACT	729
	Arg Leu Phe Gly Leu Asp Gly Asn Val Gly Thr Ala Glu Glu Asp Thr	
	230 235 240	
35	GAA CGG CAC ACA GCG CAC GAT GTG AAC CGT AAC ATG CAC ACA CTA TTA	777
	Glu Arg His Thr Ala His Asp Val Asn Arg Asn Met His Thr Leu Leu	
	245 250 255	
40	GGG GTC CGC CAG TGA	792
	Gly Val Arg Gln	
	260	

## (4) INFORMATION FOR SEQ ID NO: 3:

- (i) SEQUENCE CHARACTERISTICS:
- 40 (A) LENGTH: 793
- (B) TYPE: Nucleic Acid
- (C) STRANDEDNESS: Double
- (D) TOPOLOGY: Circular
- (ii) MOLECULE TYPE: cDNA to genomic RNA
- 45 (iii) HYPOTHETICAL: No
- (iv) ANTI-SENSE: No
- (v) FRAGMENT TYPE: N/A
- (vi) ORIGINAL SOURCE:
- (A) ORGANISM: Tobacco Etch Virus
- 50 (B) STRAIN: Highly Aphid Transmitted
- (C) INDIVIDUAL ISOLATE: N/A
- (vii) IMMEDIATE SOURCE:
- (A) LIBRARY: No
- (B) CLONE: pTC:RC
- 55 (viii) POSITION IN GENOME: N/A

(ix)

## FEATURE:

- 5 (A) NAME/KEY: Mutation of AGT-GGC  
(Ser-Gly) to ATG-GCC (Met-Ser)
- (B) LOCATION: Nucleotides 1-6 of SEQ  
ID NO. 3 (corresponding to nucleotides  
8518-8523 of SEQ ID NO. 1)
- (A) NAME/KEY: Frameshift mutation  
(insertion of T) producing stop codon
- 10 (B) LOCATION: Nucleotide 13 of SEQ ID  
No. 3 (corresponding to position  
between nucleotides 8529 and 8530 of  
SEQ. ID No. 1)
- 15 (D) OTHER INFORMATION: SEQ ID No: 3 is  
the modified Tobacco Etch Virus coat  
protein gene present in pTC:RC.

(x)

## PUBLICATION INFORMATION:

- (A) AUTHORS: J. A. Lindbo and  
W. G. Dougherty
- 20 (B) TITLE: Pathogen-Derived Resistance to  
a Potyvirus: Immune and Resistant  
Phenotypes in Transgenic Tobacco  
Expressing Altered Forms of a  
Potyvirus Coat Protein Nucleotide  
Sequence
- 25 (C) JOURNAL: Molecular Plant-Microbe  
Interactions
- (D) VOLUME: 5
- (E) ISSUE: 2
- (F) PAGES: 144-153
- 30 (A) AUTHORS: J. A. Lindbo and  
W. G. Dougherty
- 35 (B) TITLE: Untranslatable Transcripts of  
the Tobacco Etch Virus Coat Protein  
Gene Sequence Can Interfere with  
Tobacco Etch Virus Replication in  
Transgenic Plants and Protoplasts
- (C) JOURNAL: Virology
- (D) VOLUME: 189
- 40 (E) ISSUE: --
- (F) PAGES: 725-733

(xi)

## SEQUENCE DESCRIPTION: SEQ ID NO: 3:

45 GTG TGA TGA TGGTGCTAGC GCTGGTAAGA AGAAAGATCA AAAGGATGAT 58  
Val Met Ser Thr

	AAAGTCGCTG AGCAGGCTTC AAAGGATAGG GATGTTAATG CTGGAACTTC	108
	AGGAACATTC TCAGTTCCAC GAATAAATGC TATGGCCACA AA <del>A</del> CTTCAAT	158
5	ATCCAAGGAT GAGGGGAGAG GTGGTTGTAA ACTTGAATCA CCTTTTAGGA	208
	TACAAGCCAC AGCAAATTGA TTTGTCAAAT GCTCGAGCCA CACATGAGCA	258
	GTTTGCCGCG TGSCATCAGG CAGTGATGAC AGCCTATGGA GTGAATGAAG	308
10	AGCAAATGAA AATATTGCTA AATGGATTTA TGGTGTGGTG CATAGAAAAT	358
	GGGACTTCCC CAAATTTGAA CGGAACTTGG GTTATGATGG ATGGTGAGGA	408
15	TCAAGTTTCA TACCCGCTGA AACCAATGGT TGA <del>A</del> AACGCG CAGCCAACAC	458
	TGAGGCAAAT TATGACACAC TTCAGTGACC TGGCTGAAGC GTATATTGAG	508
	ATGAGGAATA GGGAGCGACC ATACATGCCT AGGTATGGTC TACAGAGAAA	558
20	CATTACAGAC ATGAGTTTGT CACGCTATGC GTTCGACTTC TATGAGCTAA	608
	CTTCA <del>A</del> AAAC ACCTGTTAGA GCGAGGGAGG CGCATATGCA AATGAAAGCT	658
25	GCTGCACTAC GAAACAGTGG AACTAGGT <del>T</del> A TTTGGTCTTG ATGGCAACGT	708
	GGGTACTGCA GAGGAAGACA CTGAACGGCA CACAGCGCAC GATGTGAACC	758
30	GTAACATGCA CACACTATTA GGGGTCCGCC AGTGA	793

## (5) INFORMATION FOR SEQ ID NO: 4

## (i) SEQUENCE CHARACTERISTICS:

- 35 (A) LENGTH: 792  
 (B) TYPE: Nucleic acid  
 (C) STRANDEDNESS: Double  
 (D) TOPOLOGY: Circular

(ii) MOLECULE TYPE: cDNA to genomic RNA

(iii) HYPOTHETICAL: No

40 (iv) ANTI-SENSE: Yes

(v) FRAGMENT TYPE: N/A

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Tobacco Etch Virus  
 (B) STRAIN: Highly Aphid Transmitted  
 45 (C) INDIVIDUAL ISOLATE: N/A

(vii) IMMEDIATE SOURCE:

- (A) LIBRARY: No  
 (B) CLONE: pTC:AS

(viii) POSITION IN GENOME: N/A

50 (ix) FEATURE:

- (A) NAME/KEY: --  
 (B) LOCATION: --  
 (C) IDENTIFICATION METHOD: --

- (D) OTHER INFORMATION: SEQ ID No. 4 is the modified Tobacco Etch Virus Coat protein gene present in pTC:AS. It is the inverse complement of SEQ ID No. 2.

5 (x) PUBLICATION INFORMATION:

- (A) AUTHORS: J. A. Lindbo and W. G. Dougherty
- (B) TITLE: Untranslatable Transcripts of the Tobacco Etch Virus Coat Protein Gene Sequence Can Interfere with Tobacco Etch Virus Replication in Transgenic Plants and Protoplasts
- (C) JOURNAL: Virology
- (D) VOLUME: 189
- (E) ISSUE: --
- (F) PAGES: 725-733

- (A) AUTHORS: J. A. Lindbo and W. G. Dougherty
- (B) TITLE: Pathogen-Derived Resistance to a Potyvirus: Immune and Resistant Phenotypes in Transgenic Tobacco Expressing Altered Forms of a Potyvirus Coat Protein Nucleotide Sequence
- (C) JOURNAL: Molecular Plant-Microbe Interactions
- (D) VOLUME: 5
- (E) ISSUE: 2
- (F) PAGES: 144-153

30 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

	TCACGTGGCGG ACCCCTAATA GTGTGTGCAT GTTACGGTTC ACATCGTGCG CTGTGTGCGG	60
	TTCAGTGTCT TCCTCTGCAG TACCCACGTT GCCATCAAGA CCAATAACC TAGTCCACT	120
	GTTTCGTACT GCAGCAGCTT TCATTGGCAT ATGCGCCTCC CTCGCTCTAA CAGGTGTTTT	180
	TGAAGTTAGC TCATAGAAGT CGAACGCATA GCGTGACAAA CTCATGTCTG TAATGTTTCT	240
35	CTGTAGACCA TACCTAGGCA TGTATGGTCC CTCCTATTTC CTCATCTCAA TATACGCTTC	300
	AGCCAGGTCA CTGAAGTGTG TCATAATTG CCTCAGTGTT GGCTGCGCGT TTTCRAACCAT	360
	TGGTTTCAGC GGGTATGAAA CTTGATCCTC ACCATCCATC ATAACCCAAG TTCCGTTCAA	420
	ATTTGGGGAA GTCCCATTTT CTATGCACCA CACCATAAAT CCATTAGCA ATATTTCAT	480
	TTGCTCTTCA TTCACTCCAT AGGCTGTCTC CACTGCCTCA TGCCACGCGG CAAACTGCTC	540
40	ATGTGTGGCT CGAGCATTTG ACAAATCAAT TTGCTGTGGC TTGTATCCTA AAAGGTGATT	600
	CAAGTTTACA ACCACCTCTC CCGTCATCCT TGGATATTCA AGTTTTGTGG CCATAGCATT	660
	TATTCGTGGA ACTGAGAATG TTCTGGAAGT TCCAGCATTA ACATCCCTAT CTTTTGAAGC	720
	CTGCTCAGCG ACTTTATCAT CTTTTGATC TTTCTTCTTA CCAGCGTCAG CACCAGCATC	780
	CACAGTGCCC AT	792

CLAIMS

1. A plant-transformation vector comprising a DNA molecule that includes a gene derived, in part, from a plant virus RNA molecule, wherein the gene is mutated to encode an untranslatable plus sense RNA molecule.  
5
2. The vector of claim 1 wherein the gene is derived, in part, from potyvirus RNA.
3. The vector of claim 2 wherein the potyvirus is Tobacco Etch Virus.
- 10 4. The vector of claim 2 wherein the gene is derived, in part, from a coat protein gene of a potyvirus.
5. The vector of claim 4 wherein the gene is derived, in part, from the coat protein gene of Tobacco Etch Virus.  
15
6. A bacterial cell containing the vector of claim 1.
7. The bacterial cell of claim 8 wherein the bacterial cell is an *Agrobacterium tumefaciens* cell.
- 20 8. A transformed plant cell comprising a heterologous DNA chromosomal insert that includes a gene derived from a plant virus RNA molecule, wherein the gene is mutated to encode an untranslatable plus sense RNA molecule.
- 25 9. The plant cell of claim 8 wherein the gene is derived from potyvirus RNA.
10. The plant cell of claim 9 wherein the potyvirus is Tobacco Etch Virus.
11. The plant cell of claim 10 wherein the gene is derived from a coat protein gene of a potyvirus.  
30
12. The plant cell of claim 10 wherein the gene is derived from the coat protein gene of Tobacco Etch Virus and the plant cell is a tobacco plant cell.
13. A differentiated plant comprising transformed plant cells of claim 8.  
35
14. A differentiated plant comprising transformed plant cells of claim 9.

15. A differentiated plant comprising transformed plant cells of claim 10.

16. A differentiated plant comprising transformed plant cells of claim 11.

5 17. A differentiated plant comprising transformed plant cells of claim 12.

18. A recombinant gene comprising:  
control regions which regulate transcription of the  
gene; and

10 a region, derived from a plant virus,  
mutated so as to render the RNA transcribed from the  
gene untranslatable.

19. The recombinant gene of claim 18 wherein  
the plant virus is a potyvirus.

15 20. The recombinant gene of claim 19 wherein  
the virus-derived region is derived from the region of  
the viral genome encoding a coat protein.

21. The recombinant gene of claim 20 wherein  
the potyvirus is Tobacco Etch Virus.

20 22. A method of producing plants with a  
reduced susceptibility to viral infection, comprising:  
forming a recombinant gene derived, in  
part, from viral RNA wherein the gene is mutated to  
encode an untranslatable plus sense RNA molecule; and  
25 transforming plants with the recombinant  
gene.

23. The method of claim 22 wherein the method  
of producing plants comprises:

30 constructing a recombinant gene comprising  
a region of a viral genome capable of being transcribed  
in a plant;

mutating the recombinant gene to encode an  
untranslatable plus sense RNA molecule;

35 cloning the recombinant untranslatable  
gene into a plant transformation vector;

transforming plant cells with the  
transformation vector; and

culturing transformed cells under conditions suitable for regeneration of transformed plants.

5           24. The method of claim 23 wherein the viral genome is a potyvirus genome.

          25. The method of claim 24 wherein the region of the viral genome encodes a coat protein.

          26. The method of claim 25 wherein the viral genome is the Tobacco Etch Virus genome.

10           27. The method of claim 26 wherein the plants are tobacco plants.

NAAATAACAA ATCTCAACAC AACATATACA AAACAAACGA ATCTCAAGCA ATCAAGCATT	60
CTACTTCTAT TGCAGCAATT TAAATCATTT CTTTAAAGC AAAAGCAATT TTCTGAAAT	120
TTTCACCATT TACGAACGAT AGCA ATG GCA CTG ATC TTT GGC ACA GTC AAC GCT	174
Met Ala Leu Ile Phe Gly Thr Val Asn Ala	10
1 5	
AAC ATC CTG AAG GAA GTG TTC GGT GGA GCT CGT ATG GCT TGC GTT ACC	222
Asn Ile Leu Lys Glu Val Phe Gly Gly Ala Arg Met Ala Cys Val Thr	25
15 20	
AGC GCA CAT ATG GCT GGA GCG AAT GGA AGC ATT TTG AAG AAG GCA GAA	270
Ser Ala His Met Ala Gly Ala Asn Gly Ser Ile Leu Lys Lys Ala Glu	40
30 35	
GAG ACC TCT CGT GCA ATC ATG CAC AAA CCA GTG ATC TTC GGA GAA GAC	318
Glu Thr Ser Arg Ala Ile Met His Lys Pro Val Ile Phe Gly Glu Asp	55
45 50	
TAC ATT ACC GAG GCA GAC TTG CCT TAC ACA CCA CTC CAT TTA GAG GTC	366
Tyr Ile Thr Glu Ala Asp Leu Pro Tyr Thr Pro Leu His Leu Glu Val	70
60 65	
GAT GCT GAA ATG GAG CGG ATG TAT TAT CTT GGT CGT CGC GCG CTC ACC	414
Asp Ala Glu Met Glu Arg Met Tyr Tyr Leu Gly Arg Arg Ala Leu Thr	90
75 80 85 90	
CAT GGC AAG AGA CGC AAA GTT TCT GTG AAT AAC AAG AGG AAC AGG AGA	462
His Gly Lys Arg Arg Lys Val Ser Val Asn Asn Lys Arg Asn Arg Arg	105
95 100 105	
AGG AAA GTG GCC AAA ACG TAC GTG GGG CGT GAT TCC ATT GTT GAG AAG	510
Arg Lys Val Ala Lys Thr Tyr Val Gly Arg Asp Ser Ile Val Glu Lys	120
110 115 120	
ATT GTA GTG CCC CAC ACC GAG AGA AAG GTT GAT ACC ACA GCA GCA GTG	558
Ile Val Val Pro His Thr Glu Arg Lys Val Asp Thr Thr Ala Ala Val	135
125 130 135	
GAA GAC ATT TGC AAT GAA GCT ACC ACT CAA CTT GTG CAT AAT AGT ATG	606
Glu Asp Ile Cys Asn Glu Ala Thr Thr Gln Leu Val His Asn Ser Met	150
140 145 150	
CCA AAG CGT AAG AAG CAG AAA AAC TTC TTG CCC GCC ACT TCA CTA AGT	654
Pro Lys Arg Lys Lys Gln Lys Asn Phe Leu Pro Ala Thr Ser Leu Ser	170
155 160 165 170	
AAC GTG TAT GCC CAA ACT TGG AGC ATA GTG CGC AAA CGC CAT ATG CAG	702
Asn Val Tyr Ala Gln Thr Trp Ser Ile Val Arg Lys Arg His Met Gln	185
175 180 185	
GTG GAG ATC ATT AGC AAG AAG AGC GTC CGA GCG AGG GTC AAG AGA TTT	750
Val Glu Ile Ile Ser Lys Lys Ser Val Arg Ala Arg Val Lys Arg Phe	200
190 195 200	
GAG GGC TCG GTG CAA TTG TTC GCA AGT GTG CGT CAC ATG TAT GGC GAG	798
Glu Gly Ser Val Gln Leu Phe Ala Ser Val Arg His Met Tyr Gly Glu	215
205 210 215	
AGG AAA AGG GTG GAC TTA CGT ATT GAC AAC TGG CAG CAA GAG ACA CTT	846
Arg Lys Arg Val Asp Leu Arg Ile Asp Asn Trp Gln Gln Glu Thr Leu	230
220 225 230	

FIG. 1

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CTA GAC CTT GCT AAA AGA TTT AAG AAT GAG AGA GTG GAT CAA TCG AAG Leu Asp Leu Ala Lys Arg Phe Lys Asn Glu Arg Val Asp Gln Ser Lys 235 240 245 250	894
CTC ACT TTT GGT TCA AGT GGC CTA GTT TTG AGG CAA GGC TCG TAC GGA Leu Thr Phe Gly Ser Ser Gly Leu Val Leu Arg Gln Gly Ser Tyr Gly 255 260 265	942
CCT GCG CAT TGG TAT CGA CAT GGT ATG TTC ATT GTA CGC GGT CGG TCG Pro Ala His Trp Tyr Arg His Gly Met Phe Ile Val Arg Gly Arg Ser 270 275 280	990
GAT GGG ATG TTG GTG GAT GCT CGT GCG AAG GTA ACG TTC GCT GTT TGT Asp Gly Met Leu Val Asp Ala Arg Ala Lys Val Thr Phe Ala Val Cys 285 290 295	1038
CAC TCA ATG ACA CAT TAT AGC GAC AAA TCA ATC TCT GAG GCA TTC TTC His Ser Met Thr His Tyr Ser Asp Lys Ser Ile Ser Glu Ala Phe Phe 300 305 310	1086
ATA CCA TAC TCT AAG AAA TTC TTG GAG TTG AGA CCA GAT GGA ATC TCC Ile Pro Tyr Ser Lys Lys Phe Leu Glu Leu Arg Pro Asp Gly Ile Ser 315 320 325 330	1134
CAT GAG TGT ACA AGA GGA GTA TCA GTT GAG CGG TGC GGT GAG GTG GCT His Glu Cys Thr Arg Gly Val Ser Val Glu Arg Cys Gly Glu Val Ala 335 340 345	1182
GCA ATC CTG ACA CAA GCA CTT TCA CCG TGT GGT AAG ATC ACA TGC AAA Ala Ile Leu Thr Gln Ala Leu Ser Pro Cys Gly Lys Ile Thr Cys Lys 350 355 360	1230
CGT TGC ATG GTT GAA ACA CCT GAC ATT GTT GAG GGT GAG TCG GGA GAA Arg Cys Met Val Glu Thr Pro Asp Ile Val Glu Gly Glu Ser Gly Glu 365 370 375	1278
AGT GTC ACC AAC CAA GGT AAG CTC CTA GCA ATG CTG AAA GAA CAG TAT Ser Val Thr Asn Gln Gly Lys Leu Leu Ala Met Leu Lys Glu Gln Tyr 380 385 390	1326
CCA GAT TTC CCA ATG GCC GAG AAA CTA CTC ACA AGG TTT TTG CAA CAG Pro Asp Phe Pro Met Ala Glu Lys Leu Leu Thr Arg Phe Leu Gln Gln 395 400 405 410	1374
AAA TCA CTA GTA AAT ACA AAT TTG ACA GCC TGC GTG AGC GTC AAA CAA Lys Ser Leu Val Asn Thr Asn Leu Thr Ala Cys Val Ser Val Lys Gln 415 420 425	1422
CTC ATT GGT GAC CGC AAA CAA GCT CCA TTC ACA CAC GTA CTG GCT GTC Leu Ile Gly Asp Arg Lys Gln Ala Pro Phe Thr His Val Leu Ala Val 430 435 440	1470
AGC GAA ATT CTG TTT AAA GGC AAT AAA CTA ACA GGG GCT GAT CTC GAA Ser Glu Ile Leu Phe Lys Gly Asn Lys Leu Thr Gly Ala Asp Leu Glu 445 450 455	1518
GAG GCA AGC ACA CAT ATG CTT GAA ATA GCA AGG TTC TTG AAC AAT CGC Glu Ala Ser Thr His Met Leu Glu Ile Ala Arg Phe Leu Asn Asn Arg 460 465 470	1566
ACT GAA AAT ATG CGC ATT GGC CAC CTT GGT TCT TTC ACA AAT AAA ATC Thr Glu Asn Met Arg Ile Gly His Leu Gly Ser Phe Arg Asn Lys Ile 475 480 485 490	1614

FIG. 1

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TCA	TCG	AAG	GCC	CAT	GTG	AAT	AAC	GCA	CTC	ATG	TGT	GAT	AAT	CAA	CTT	1662
Ser	Ser	Lys	Ala	His	Val	Asn	Asn	Ala	Leu	Met	Cys	Asp	Asn	Gln	Leu	
				495					500					505		
GAT	CAG	AAT	GGG	AAT	TTT	ATT	TGG	GGA	CTA	AGG	GGT	GCA	CAC	GCA	AAG	1710
Asp	Gln	Asn	Gly	Asn	Phe	Ile	Trp	Gly	Leu	Arg	Gly	Ala	His	Ala	Lys	
			510					515					520			
AGG	TTT	CTT	AAA	GGA	TTT	TTC	ACT	GAG	ATT	GAC	CCA	AAT	GAA	GGA	TAC	1758
Arg	Phe	Leu	Lys	Gly	Phe	Phe	Thr	Glu	Ile	Asp	Pro	Asn	Glu	Gly	Tyr	
		525					530					535				
GAT	AAG	TAT	GTT	ATC	AGG	AAA	CAT	ATC	AGG	GGT	AGC	AGA	AAG	CTA	GCA	1806
Asp	Lys	Tyr	Val	Ile	Arg	Lys	His	Ile	Arg	Gly	Ser	Arg	Lys	Leu	Ala	
		540				545					550					
ATT	GGC	AAT	TTG	ATA	ATG	TCA	ACT	GAC	TTC	CAG	ACG	CTC	AGG	CAA	CAA	1854
Ile	Gly	Asn	Leu	Ile	Met	Ser	Thr	Asp	Phe	Gln	Thr	Leu	Arg	Gln	Gln	
555					560					565					570	
ATT	CAA	GGC	GAA	ACT	ATT	GAG	CGT	AAA	GAA	ATT	GGG	AAT	CAC	TGC	ATT	1902
Ile	Gln	Gly	Glu	Thr	Ile	Glu	Arg	Lys	Glu	Ile	Gly	Asn	His	Cys	Ile	
				575					580					585		
TCA	ATG	CGG	AAT	GGT	AAT	TAC	GTG	TAC	CCA	TGT	TGT	TGT	GTT	ACT	CTT	1950
Ser	Met	Arg	Asn	Gly	Asn	Tyr	Val	Tyr	Pro	Cys	Cys	Cys	Val	Thr	Leu	
			590					595					600			
GAA	GAT	GGT	AAG	GCT	CAA	TAT	TCG	GAT	CTA	AAG	CAC	CCA	ACG	AAG	AGA	1998
Glu	Asp	Gly	Lys	Ala	Gln	Tyr	Ser	Asp	Leu	Lys	His	Pro	Thr	Lys	Arg	
		605					610					615				
CAT	CTG	GTC	ATT	GGC	AAC	TCT	GGC	GAT	TCA	AAG	TAC	CTA	GAC	CTT	CCA	2046
His	Leu	Val	Ile	Gly	Asn	Ser	Gly	Asp	Ser	Lys	Tyr	Leu	Asp	Leu	Pro	
		620				625						630				
GTT	CTC	AAT	GAA	GAG	AAA	ATG	TAT	ATA	GCT	AAT	GAA	GGT	TAT	TGC	TAC	2094
Val	Leu	Asn	Glu	Glu	Lys	Met	Tyr	Ile	Ala	Asn	Glu	Gly	Tyr	Cys	Tyr	
635					640					645					650	
ATG	AAC	ATT	TTC	TTT	GCT	CTA	CTA	GTG	AAT	GTC	AAG	GAA	GAG	GAT	GCA	2142
Met	Asn	Ile	Phe	Phe	Ala	Leu	Leu	Val	Asn	Val	Lys	Glu	Glu	Asp	Ala	
				655					660					665		
AAG	GAC	TTC	ACC	AAG	TTT	ATA	AGG	GAC	ACA	ATT	GTT	CCA	AAG	CTT	GGA	2190
Lys	Asp	Phe	Thr	Lys	Phe	Ile	Arg	Asp	Thr	Ile	Val	Pro	Lys	Leu	Gly	
			670					675					680			
GCG	TGG	CCA	ACA	ATG	CAA	GAT	GTT	GCA	ACT	GCA	TGC	TAC	TTA	CTT	TCC	2238
Ala	Trp	Pro	Thr	Met	Gln	Asp	Val	Ala	Thr	Ala	Cys	Tyr	Leu	Leu	Ser	
		685					690					695				
ATT	CTT	TAC	CCA	GAT	GTC	CTG	AGA	GCT	GAA	CTA	CCC	AGA	ATT	TTG	GTT	2286
Ile	Leu	Tyr	Pro	Asp	Val	Leu	Arg	Ala	Glu	Leu	Pro	Arg	Ile	Leu	Val	
		700				705					710					
GAT	CAT	GAC	AAC	AAA	ACA	ATG	CAT	GTT	TTG	GAT	TCG	TAT	GGG	TCT	AGA	2334
Asp	His	Asp	Asn	Lys	Thr	Met	His	Val	Leu	Asp	Ser	Tyr	Gly	Ser	Arg	
		715			720					725					730	
ACG	ACA	GGA	TAC	CAC	ATG	TTG	AAA	ATG	AAC	ACA	ACA	TCC	CAG	CTA	ATT	2382
Thr	Thr	Gly	Tyr	His	Met	Leu	Lys	Met	Asn	Thr	Thr	Ser	Gln	Leu	Ile	
				735					740					745		

FIG. 1

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GAA TTC GTT CAT TCA GGT TTG GAA TCC GAA ATG AAA ACT TAC AAT GTT Glu Phe Val His Ser Gly Leu Glu Ser Glu Met Lys Thr Tyr Asn Val 750 755 760	2430
GGA GGG ATG AAC CGA GAT GTG GTC ACA CAA GGT GCA ATT GAG ATG TTG Gly Gly Met Asn Arg Asp Val Val Thr Gln Gly Ala Ile Glu Met Leu 765 770 775	2478
ATC AAG TCT ATA TAC AAA CCA CAT CTC ATG AAG CAG TTA CTT GAG GAA Ile Lys Ser Ile Tyr Lys Pro His Leu Met Lys Gln Leu Leu Glu Glu 780 785 790	2526
GAG CCA TAC ATA ATT GTC CTG GCA ATA GTC TCC CCT TCA ATT TTA ATT Glu Pro Tyr Ile Ile Val Leu Ala Ile Val Ser Pro Ser Ile Leu Ile 795 800 805 810	2574
GCC ATG TAC AAC TCT GGA ACT TTT GAG CAG GCG TTA CAA ATG TGG TTG Ala Met Tyr Asn Ser Gly Thr Phe Glu Gln Ala Leu Gln Met Trp Leu 815 820 825	2622
CCA AAT ACA ATG AGG TTA GCT AAC CTC GCT GCC ATC TTG TCA GCC TTA Pro Asn Thr Met Arg Leu Ala Asn Leu Ala Ala Ile Leu Ser Ala Leu 830 835 840	2670
GCG CAA AAG TTA ACT TTG GCA GAT TTG TTC GTC CAG CAG CGT AAT TTG Ala Gln Lys Leu Thr Leu Ala Asp Leu Phe Val Gln Gln Arg Asn Leu 845 850 855	2718
ATT AAT GAG TAT GCG CAG GTA ATT TTG GAC AAT CTG ATT GAC GGT GTC Ile Asn Glu Tyr Ala Gln Val Ile Leu Asp Asn Leu Ile Asp Gly Val 860 865 870	2766
AGG GTT AAT CAT TCG CTA TCC CTA GCA ATG GAA ATT GTT ACT ATT AAG Arg Val Asn His Ser Leu Ser Leu Ala Met Glu Ile Val Thr Ile Lys 875 880 885 890	2814
CTG GCC ACC CAA GAG ATG GAC ATG GCG TTG AGG GAA GGT GGC TAT GCT Leu Ala Thr Gln Glu Met Asp Met Ala Leu Arg Glu Gly Gly Tyr Ala 895 900 905	2862
GTG ACC TCT GAA AAG GTG CAT GAA ATG TTG GAA AAA AAC TAT GTA AAG Val Thr Ser Glu Lys Val His Glu Met Leu Glu Lys Asn Tyr Val Lys 910 915 920	2910
GCT TTG AAG GAT GCA TGG GAC GAA TTA ACT TGG TTG GAA AAA TTC TCC Ala Leu Lys Asp Ala Trp Asp Glu Leu Thr Trp Leu Glu Lys Phe Ser 925 930 935	2958
GCA ATC AGG CAT TCA AGA AAG CTC TTG AAA TTT GGG CGA AAG CCT TTA Ala Ile Arg His Ser Arg Lys Leu Leu Lys Phe Gly Arg Lys Pro Leu 940 945 950	3006
ATC ATG AAA AAC ACC GTA GAT TGC GGC GGA CAT ATA GAC TTG TCT GTG Ile Met Lys Asn Thr Val Asp Cys Gly Gly His Ile Asp Leu Ser Val 955 960 965 970	3054
AAA TCG CTT TTC AAG TTC CAC TTG GAA CTC CTG AAG GGA ACC ATC TCA Lys Ser Leu Phe Lys Phe His Leu Glu Leu Leu Lys Gly Thr Ile Ser 975 980 985	3102
AGA GCC GTA AAT GGT GGC GCA AGA AAG GTA AGA GTA GCG AAG AAT GCC Arg Ala Val Asn Gly Gly Ala Arg Lys Val Arg Val Ala Lys Asn Ala 990 995 1000	3150

FIG. 1

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ATG ACA AAA GGG GTT TTT CTC AAA ATC TAC AGC ATG CTT CCT GAC GTC Met Thr Lys Gly Val Phe Leu Lys Ile Tyr Ser Met Leu Pro Asp Val 1005 1010 1015	3198
TAC AAG TTT ATC ACA GTC TCG AGT GTC CTT TCC TTG TTG TTG ACA TTC Tyr Lys Phe Ile Thr Val Ser Ser Val Leu Ser Leu Leu Leu Thr Phe 1020 1025 1030	3246
TTA TTT CAA ATT GAC TGC ATG ATA AGG GCA CAC CGA GAG GCG AAG GTT Leu Phe Gln Ile Asp Cys Met Ile Arg Ala His Arg Glu Ala Lys Val 1035 1040 1045 1050	3294
GCT GCA CAG TTG CAG AAA GAG AGC GAG TGG GAC AAT ATC ATC AAT AGA Ala Ala Gln Leu Gln Lys Glu Ser Glu Trp Asp Asn Ile Ile Asn Arg 1055 1060	3342
ACT TTC CAG TAT TCT AAG CTT GAA AAT CCT ATT GGC TAT CGC TCT ACA Thr Phe Gln Tyr Ser Lys Leu Glu Asn Pro Ile Gly Tyr Arg Ser Thr 1070 1075 1080	3390
GCG GAG GAA AGA CTC CAA TCA GAA CAC CCC GAG GCT TTC GAG TAC TAC Ala Glu Glu Arg Leu Gln Ser Glu His Pro Glu Ala Phe Glu Tyr Tyr 1085 1090 1095	3438
AAG TTT TGC ATT GGA AAG GAA GAC CTC GTT GAA CAG GCA AAA CAA CCG Lys Phe Cys Ile Gly Lys Glu Asp Leu Val Glu Gln Ala Lys Gln Pro 1100 1105 1110	3486
GAG ATA GCA TAC TTT GAA AAG ATT ATA GCT TTC ATC ACA CTT GTA TTA Glu Ile Ala Tyr Phe Glu Lys Ile Ile Ala Phe Ile Thr Leu Val Leu 1115 1120 1125 1130	3534
ATG GCT TTT GAC GCT GAG CGG AGT GAT GGA GTG TTC AAG ATA CTC AAT Met Ala Phe Asp Ala Glu Arg Ser Asp Gly Val Phe Lys Ile Leu Asn 1135 1140 1145	3582
AAG TTC AAA GGA ATA CTG AGC TCA ACG GAG AGG GAG ATC ATC TAC ACG Lys Phe Lys Gly Ile Leu Ser Ser Thr Glu Arg Glu Ile Ile Tyr Thr 1150 1155 1160	3630
CAG AGT TTG GAT GAT TAC GTT ACA ACC TTT GAT GAC AAT ATG ACA ATC Gln Ser Leu Asp Asp Tyr Val Thr Thr Phe Asp Asp Asn Met Thr Ile 1165 1170 1175	3678
AAC CTC GAG TTG AAT ATG GAT GAA CTC CAC AAG ACG AGC CTT CCT GGA Asn Leu Glu Leu Asn Met Asp Glu Leu His Lys Thr Ser Leu Pro Gly 1180 1185 1190	3726
GTC ACT TTT AAG CAA TGG TGG AAC AAC CAA ATC AGC CGA GGC AAC GTG Val Thr Phe Lys Gln Trp Trp Asn Asn Gln Ile Ser Arg Gly Asn Val 1195 1200 1205 1210	3774
AAG CCA CAT TAT AGA ACT GAG GGG CAC TTC ATG GAG TTT ACC AGA GAT Lys Pro His Tyr Arg Thr Glu Gly His Phe Met Glu Phe Thr Arg Asp 1215 1220 1225	3822
ACT GCG GCA TCG GTT GCC AGC GAG ATA TCA CAC TCA CCC GCA AGA GAT Thr Ala Ala Ser Val Ala Ser Glu Ile Ser His Ser Pro Ala Arg Asp 1230 1235 1240	3870
TTT CTT GTG AGA GGT GCT GTT GGA TCT GGA AAA TCC ACA GGA CTT CCA Phe Leu Val Arg Gly Ala Val Gly Ser Gly Lys Ser Thr Gly Leu Pro 1245 1250 1255	3918

FIG. 1

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TAC CAT TTA TCA AAG AGA GGG AGA GTG TTA ATG CTT GAG CCT ACC AGA Tyr His Leu Ser Lys Arg Gly Arg Val Leu Met Leu Glu Pro Thr Arg 1260 1265 1270	3966
CCA CTC ACA GAT AAC ATG CAC AAG CAA CTG AGA AGT GAA CCA TTT AAC Pro Leu Thr Asp Asn Met His Lys Gln Leu Arg Ser Glu Pro Phe Asn 1275 1280 1285 1290	4014
TGC TTC CCA ACT TTG AGG ATG AGA GGG AAG TCA ACT TTT GGG TCA TCA Cys Phe Pro Thr Leu Arg Met Arg Gly Lys Ser Thr Phe Gly Ser Ser 1295 1300 1305	4062
CCG ATC ACA GTC ATG ACT AGT GGA TTC GCT TTA CAC CAC TTT GCA CGA Pro Ile Thr Val Met Thr Ser Gly Phe Ala Leu His His Phe Ala Arg 1310 1315 1320	4110
AAC ATA GCT GAG GTA AAA ACA TAC GAT TTT GTC ATA ATT GAT GAA TGT Asn Ile Ala Glu Val Lys Thr Tyr Asp Phe Val Ile Ile Asp Glu Cys 1325 1330 1335	4158
CAT GTG AAT GAT GCT TCT GCT ATA GCG TTT AGG AAT CTA CTG TTT GAA His Val Asn Asp Ala Ser Ala Ile Ala Phe Arg Asn Leu Leu Phe Glu 1340 1345 1350	4206
CAT GAA TTT GAA GGA AAA GTC CTC AAA GTG TCA GCC ACA CCA CCA GGT His Glu Phe Glu Gly Lys Val Leu Lys Val Ser Ala Thr Pro Pro Gly 1355 1360 1365 1370	4254
AGA GAA GTT GAA TTT ACA ACT CAG TTT CCC GTG AAA CTC AAG ATA GAA Arg Glu Val Glu Phe Thr Thr Gln Phe Pro Val Lys Leu Lys Ile Glu 1375 1380 1385	4302
GAG GCT CTT AGC TTT CAG GAA TTT GTA AGT TTA CAA GGG ACA GGT GCC Glu Ala Leu Ser Phe Gln Glu Phe Val Ser Leu Gln Gly Thr Gly Ala 1390 1395 1400	4350
AAC GCC GAT GTG ATT AGT TGT GGC GAC AAC ATA CTA GTA TAT GTT GCT Asn Ala Asp Val Ile Ser Cys Gly Asp Asn Ile Leu Val Tyr Val Ala 1405 1410 1415	4398
AGC TAC AAT GAT GTT GAT AGT CTT GGC AAG CTC CTT GTG CAA AAG GGA Ser Tyr Asn Asp Val Asp Ser Leu Gly Lys Leu Val Gln Lys Gly 1420 1425 1430	4446
TAC AAA GTG TCG AAG ATT GAT GGA AGA ACA ATG AAG AGT GGA GGA ACT Tyr Lys Val Ser Lys Ile Asp Gly Arg Thr Met Lys Ser Gly Gly Thr 1435 1440 1445 1450	4494
GAA ATA ATC ACT GAA GGT ACT TCA GTG AAA AAG CAT TTC ATA GTC GCA Glu Ile Ile Thr Glu Gly Thr Ser Val Lys Lys His Phe Ile Val Ala 1455 1460 1465	4542
ACT AAC ATT ATT GAG AAT GGT GTA ACC ATT GAC ATT GAT GTA GTT GTG Thr Asn Ile Ile Glu Asn Gly Val Thr Ile Asp Ile Asp Val Val Val 1470 1475 1480	4590
GAT TTT GGG ACT AAG GTT GTA CCA GTT TTG GAT GTG GAC AAT AGA GCG Asp Phe Gly Thr Lys Val Val Pro Val Leu Asp Val Asp Asn Arg Ala 1481 1490 1495	4638
GTG CAG TAC AAC AAA ACT GTG GTG AGT TAT GGG GAG CGC ATC CAA AAA Val Gln Tyr Asn Lys Thr Val Val Ser Tyr Gly Glu Arg Ile Gln Lys 1500 1505 1510	4686

FIG. 1

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CTC GGT AGA GTT GGG CGA CAC AAG GAA GGA GTA GCA CTT CGA ATT GGC Leu Gly Arg Val Gly Arg His Lys Glu Gly Val Ala Leu Arg Ile Gly 1515 1520 1525 1530	4734
CAA ACA AAT AAA ACA CTG GTT GAA ATT CCA GAA ATG GTT GCC ACT GAA Gln Thr Asn Lys Thr Leu Val Glu Ile Pro Glu Met Val Ala Thr Glu 1535 1540 1545	4782
GCT GCC TTT CTA TGC TTC ATG TAC AAT TTG CCA GTG ACA ACA CAG AGT Ala Ala Phe Leu Cys Phe Met Tyr Asn Leu Pro Val Thr Thr Gln Ser 1550 1555 1560	4830
GTT TCA ACC ACA CTG CTG GAA AAT GCC ACA TTA TTA CAA GCT AGA ACT Val Ser Thr Thr Leu Leu Glu Asn Ala Thr Leu Leu Gln Ala Arg Thr 1565 1570 1575	4878
ATG GCA CAG TTT GAG CTA TCA TAT TTT TAC ACA ATT AAT TTT GTG CGA Met Ala Gln Phe Glu Leu Ser Tyr Phe Tyr Thr Ile Asn Phe Val Arg 1580 1585 1590	4926
TTT GAT GGT AGT ATG CAT CCA GTC ATA CAT GAC AAG CTG AAG CGC TTT Phe Asp Gly Ser Met His Pro Val Ile His Asp Lys Leu Lys Arg Phe 1595 1600 1605 1610	4974
AAG CTA CAC ACT TGT GAG ACA TTC CTC AAT AAG TTG GCG ATC CCA AAT Lys Leu His Thr Cys Glu Thr Phe Leu Asn Lys Leu Ala Ile Pro Asn 1615 1620 1625	5022
AAA GGC TTA TCC TCT TGG CTT ACG AGT GGA GAG TAT AAG CGA CTT GGT Lys Gly Leu Ser Ser Trp Leu Thr Ser Gly Glu Tyr Lys Arg Leu Gly 1630 1635 1640	5070
TAC ATA GCA GAG GAT GCT GGC ATA AGA ATC CCA TTC GTG TGC AAA GAA Tyr Ile Ala Glu Asp Ala Gly Ile Arg Ile Pro Phe Val Cys Lys Glu 1645 1650 1655	5118
ATT CCA GAC TCC TTG CAT GAG GAA ATT TGG CAC ATT GTA GTC GCC CAT Ile Pro Asp Ser Leu His Glu Glu Ile Trp His Ile Val Val Ala His 1660 1665 1670	5166
AAA GGT GAC TCG GGT ATT GGG AGG CTC ACT AGC GTA CAG GCA GCA AAG Lys Gly Asp Ser Gly Ile Gly Arg Leu Thr Ser Val Gln Ala Ala Lys 1675 1680 1685 1690	5214
GTT GTT TAT ACT CTG CAA ACG GAT GTG CAC TCA ATT GCG AGG ACT CTA Val Val Tyr Thr Leu Gln Thr Asp Val His Ser Ile Ala Arg Thr Leu 1695 1700 1705	5262
GCA TGC ATC AAT AGA CGC ATA GCA GAT GAA CAA ATG AAG CAG AGT CAT Ala Cys Ile Asn Arg Arg Ile Ala Asp Glu Gln Met Lys Gln Ser His 1710 1715 1720	5310
TTT GAA GCC GCA ACT GGG AGA GCA TTT TCC TTC ACA AAT TAC TCA ATA Phe Glu Ala Ala Thr Gly Arg Ala Phe Ser Phe Thr Asn Tyr Ser Ile 1725 1730 1735	5358
CAA AGC ATA TTT GAC ACG CTG AAA GCA AAT TAT GCT ACA AAG CAT ACG Gln Ser Ile Phe Asp Thr Leu Lys Ala Asn Tyr Ala Thr Lys His Thr 1740 1745 1750	5406
AAA GAA AAT ATT GCA GTG CTT CAG CAG GCA AAA GAT CAA TTG CTA GAG Lys Glu Asn Ile Ala Val Leu Gln Gln Ala Lys Asp Gln Leu Leu Glu 1755 1760 1765 1770	5454

FIG. 1

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TTT TCG AAC CTA GCA AAG GAT CAA GAT GTC ACG GGT ATC ATC CAA GAC Phe Ser Asn Leu Ala Lys Asp Gln Asp Val Thr Gly Ile Ile Gln Asp 1775 1780 1785	5502
TTC AAT CAC CTG GAA ACT ATC TAT CTC CAA TCA GAT AGC GAA GTG GCT Phe Asn His Leu Glu Thr Ile Tyr Leu Gln Ser Asp Ser Glu Val Ala 1790 1795 1800	5550
AAG CAT CTG AAG CTT AAA AGT CAC TGG AAT AAA AGC CAA ATC ACT AGG Lys His Leu Lys Leu Lys Ser His Trp Asn Lys Ser Gln Ile Thr Arg 1805 1810 1815	5598
GAC ATC ATA ATA GCT TTG TCT GTG TTA ATT GGT GGT GGA TGG ATG CTT Asp Ile Ile Ile Ala Leu Ser Val Leu Ile Gly Gly Gly Trp Met Leu 1820 1825 1830	5646
GCA ACG TAC TTC AAG GAC AAG TTC AAT GAA CCA GTC TAT TTC CAA GGG Ala Thr Tyr Phe Lys Asp Lys Phe Asn Glu Pro Val Tyr Phe Gln Gly 1835 1840 1845 1850	5694
AAG AAG AAT CAG AAG CAC AAG CTT AAG ATG AGA GAG GCG CGT GGG GCT Lys Lys Asn Gln Lys His Lys Leu Lys Met Arg Glu Ala Arg Gly Ala 1855 1860 1865	5742
AGA GGG CAA TAT GAG GTT GCA GCG GAG CCA GAG GCG CTA GAA CAT TAC Arg Gly Gln Tyr Glu Val Ala Ala Glu Pro Glu Ala Leu Glu His Tyr 1870 1875 1880	5790
TTT GGA AGC GCA TAT AAT AAC AAA GGA AAG CGC AAG GGC ACC ACG AGA Phe Gly Ser Ala Tyr Asn Asn Lys Gly Lys Arg Lys Gly Thr Thr Arg 1885 1890 1895	5838
GGA ATG GGT GCA AAG TCT CGG AAA TTC ATA AAC ATG TAT GGG TTT GAT Gly Met Gly Ala Lys Ser Arg Lys Phe Ile Asn Met Tyr Gly Phe Asp 1900 1905 1910	5886
CCA ACT GAT TTT TCA TAC ATT AGG TTT GTG GAT CCA TTG ACA GGT CAC Pro Thr Asp Phe Ser Tyr Ile Arg Phe Val Asp Pro Leu Thr Gly His 1915 1920 1925 1930	5934
ACT ATT GAT GAG TCC ACA AAC GCA CCT ATT GAT TTA GTG CAG CAT GAG Thr Ile Asp Glu Ser Thr Asn Ala Pro Ile Asp Leu Val Gln His Glu 1935 1940 1945	5982
TTT GGA AAG GTT AGA ACA CGC ATG TTA ATT GAC GAT GAG ATA GAG CCT Phe Gly Lys Val Arg Thr Arg Met Leu Ile Asp Asp Glu Ile Glu Pro 1950 1955 1960	6030
CAA AGT CTT AGC ACC CAC ACC ACA ATC CAT GCT TAT TTG GTG AAT AGT Gln Ser Leu Ser Thr His Thr Thr Ile His Ala Tyr Leu Val Asn Ser 1965 1970 1975	6078
GGC ACG AAG AAA GTT CTT AAG GTT GAT TTA ACA CCA CAC TCG TCG CTA Gly Thr Lys Lys Val Leu Lys Val Asp Leu Thr Pro His Ser Ser Leu 1980 1985 1990	6126
CGT GCG AGT GAG AAA TCA ACA GCA ATA ATG GGA TTT CCT GAA AGG GAG Arg Ala Ser Glu Lys Ser Thr Ala Ile Met Gly Phe Pro Glu Arg Glu 1995 2000 2005 2010	6174
AAT GAA TTG CGT CAA ACC GGC ATG GCA GTG CCA GTG GCT TAT GAT CAA Asn Glu Leu Arg Gln Thr Gly Met Ala Val Pro Val Ala Tyr Asp Gln 2015 2020 2025	6222

FIG. 1

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TTG CCA CCA AAG AAT GAG GAC TTG ACG TTT GAA GGA GAA AGC TTG TTT Leu Pro Pro Lys Asn Glu Asp Leu Thr Phe Glu Gly Glu Ser Leu Phe 2030 2035 2040	6270
AAG GGA CCA CGT GAT TAC AAC CCG ATA TCG AGC ACC ATT TGT CAT TTG Lys Gly Pro Arg Asp Tyr Asn Pro Ile Ser Ser Thr Ile Cys His Leu 2045 2050 2055	6318
ACG AAT GAA TCT GAT GGG CAC ACA ACA TCG TTG TAT GGT ATT GGA TTT Thr Asn Glu Ser Asp Gly His Thr Thr Ser Leu Tyr Gly Ile Gly Phe 2060 2065 2070	6366
GGT CCC TTC ATC ATT ACA AAC AAG CAC TTG TTT AGA AGA AAT AAT GGA Gly Pro Phe Ile Ile Ile Thr Asn Lys His Leu Phe Arg Arg Asn Asn Gly 2075 2080 2085 2090	6414
ACA CTG TTG GTC CAA TCA CTA CAT GGT GTA TTC AAG GTC AAG AAC ACC Thr Leu Leu Val Gln Ser Leu His Gly Val Phe Lys Val Lys Asn Thr 2095 2100 2105	6462
ACG ACT TTG CAA CAA CAC CTC ATT GAT GGG AGG GAC ATG ATA ATT ATT Thr Thr Leu Gln Gln His Leu Ile Asp Gly Arg Asp Met Ile Ile Ile 2110 2115 2120	6510
CGC ATG CCT AAG GAT TTC CCA CCA TTT CCT CAA AAG CTG AAA TTT AGA Arg Met Pro Lys Asp Phe Pro Pro Phe Pro Gln Lys Leu Lys Phe Arg 2125 2130 2135	6558
GAG CCA CAA AGG GAA GAG CGC ATA TGT CTT GTG ACA ACC AAC TTC CAA Glu Pro Gln Arg Glu Glu Arg Ile Cys Leu Val Thr Thr Asn Phe Gln 2140 2145 2150	6606
ACT AAG AGC ATG TCT AGC ATG GTG TCA GAC ACT AGT TGC ACA TTC CCT Thr Lys Ser Met Ser Ser Met Val Ser Asp Thr Ser Cys Thr Phe Pro 2155 2160 2165 2170	6654
TCA TCT GAT GGC ATA TTC TGG AAG CAT TGG ATT CAA ACC AAG GAT GGG Ser Ser Asp Gly Ile Phe Trp Lys His Trp Ile Gln Thr Lys Asp Gly 2175 2180 2185	6702
CAG TGT GGC AGT CCA TTA GTA TCA ACT AGA GAT GGG TTC ATT GTT GGT Gln Cys Gly Ser Pro Leu Val Ser Thr Arg Asp Gly Phe Ile Val Gly 2190 2195 2200	6750
ATA CAC TCA GCA TCG AAT TTC ACC AAC ACA AAC AAT TAT TTC ACA AGC Ile His Ser Ala Ser Asn Phe Thr Asn Thr Asn Asn Tyr Phe Thr Ser 2205 2210 2215	6798
GTG CCG AAA AAC TTC ATG GAA TTG TTG ACA AAT CAG GAG GCG CAG CAG Val Pro Lys Asn Phe Met Glu Leu Leu Thr Asn Gln Glu Ala Gln Gln 2220 2225 2230	6846
TGG GTT AGT GGT TGG CGA TTA AAT GCT GAC TCA GTA TTG TGG GGG GGC Trp Val Ser Gly Trp Arg Leu Asn Ala Asp Ser Val Leu Trp Gly Gly 2235 2240 2245 2250	6894
CAT AAA GTT TTC ATG AGC AAA CCT GAA GAG CCT TTT CAG CCA GTT AAG His Lys Val Phe Met Ser Lys Pro Glu Glu Pro Phe Gln Pro Val Lys 2255 2260 2265	6942
GAA GCG ACT CAA CTC ATG AAT GAA TTG GTG TAC TCG CAA GGG GAG AAG Glu Ala Thr Gln Leu Met Asn Glu Leu Val Tyr Ser Gln Gly Glu Lys 2270 2275 2280	6990

FIG. 1

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AGG AAA TGG GTC GTG GAA GCA CTG TCA GGG AAC TTG AGG CCA GTG GCT	7038
Arg Lys Trp Val Val Glu Ala Leu Ser Gly Asn Leu Arg Pro Val Ala	
2285 2290 2295	
GAG TGT CCC AGT CAG TTA GTC ACA AAG CAT GTG GTT AAA GGA AAG TGT	7086
Glu Cys Pro Ser Gln Leu Val Thr Lys His Val Val Lys Gly Lys Cys	
2300 2305 2310	
CCC CTC TTT GAG CTC TAC TTG CAG TTG AAT CCA GAA AAG GAA GCA TAT	7134
Pro Leu Phe Glu Leu Tyr Leu Gln Leu Asn Pro Glu Lys Glu Ala Tyr	
2315 2320 2325 2330	
TTT AAA CCG ATG ATG GGA GCA TAT AAG CCA AGT CGA CTT AAT AGA GAG	7182
Phe Lys Pro Met Met Gly Ala Tyr Lys Pro Ser Arg Leu Asn Arg Glu	
2335 2340 2345	
GCG TTC CTC AAG GAC ATT CTA AAA TAT GCT AGT GAA ATT GAG ATT GGG	7230
Ala Phe Leu Lys Asp Ile Leu Lys Tyr Ala Ser Glu Ile Glu Ile Gly	
2350 2355 2360	
AAT GTG GAT TGT GAC TTG CTG GAG CTT GCA ATA AGC ATG CTC GTC ACA	7278
Asn Val Asp Cys Asp Leu Leu Glu Leu Ala Ile Ser Met Leu Val Thr	
2365 2370 2375	
AAG CTC AAG GCG TTA GGA TTC CCA ACT GTG AAC TAC ATC ACT GAC CCA	7326
Lys Leu Lys Ala Leu Gly Phe Pro Thr Val Asn Tyr Ile Thr Asp Pro	
2380 2385 2390	
GAG GAA ATT TTT AGT GCA TTG AAT ATG AAA GCA GCT ATG GGA GCA CTA	7374
Glu Glu Ile Phe Ser Ala Leu Asn Met Lys Ala Ala Met Gly Ala Leu	
2395 2400 2405 2410	
TAC AAA GGC AAG AAG AAA GAA GCT CTC AGC GAG CTC ACA CTA GAT GAG	7422
Tyr Lys Gly Lys Lys Lys Glu Ala Leu Ser Glu Leu Thr Leu Asp Glu	
2415 2420 2425	
CAG GAG GCA ATG CTC AAA GCA AGT TGC CTG CGA CTG TAT ACG GGA AAG	7470
Gln Glu Ala Met Leu Lys Ala Ser Cys Leu Arg Leu Tyr Thr Gly Lys	
2430 2435 2440	
TTG GGA ATT TGG AAT GGC TCA TTG AAA GCA GAG TTG CGT CCA ATT GAG	7518
Leu Gly Ile Trp Asn Gly Ser Leu Lys Ala Glu Leu Arg Pro Ile Glu	
2445 2450 2455	
AAG GTT GAA AAC AAC AAA ACG CGA ACT TTC ACA GCA GCA CCA ATA GAC	7566
Lys Val Glu Asn Asn Lys Thr Arg Thr Phe Thr Ala Ala Pro Ile Asp	
2460 2465 2470	
ACT CTT CTT GCT GGT AAA GTT TGC GTG GAT GAT TTC AAC AAT CAA TTT	7614
Thr Leu Leu Ala Gly Lys Val Cys Val Asp Asp Phe Asn Asn Gln Phe	
2475 2480 2485 2490	
TAT GAT CTC AAC ATA AAG GCA CCA TGG ACA GTT GGT ATG ACT AAG TTT	7662
Tyr Asp Leu Asn Ile Lys Ala Pro Trp Thr Val Gly Met Thr Lys Phe	
2495 2500 2505	
TAT CAG GGG TGG AAT GAA TTG ATG GAG GCT TTA CCA AGT GGG TGG GTG	7710
Tyr Gln Gly Trp Asn Glu Leu Met Glu Ala Leu Pro Ser Gly Trp Val	
2510 2515 2520	
TAT TGT GAC GCT GAT GGT TCG CAA TTC GAC AGT TCC TTG ACT CCA TTC	7758
Tyr Cys Asp Ala Asp Gly Ser Gln Phe Asp Ser Ser Leu Thr Pro Phe	
2525 2530 2535	

FIG. 1

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CTC	ATT	AAT	GCT	GTA	TTG	AAA	GTG	CGA	CTT	GCC	TTC	ATG	GAG	GAA	TGG	7806
Leu	Ile	Asn	Ala	Val	Leu	Lys	Val	Arg	Leu	Ala	Phe	Met	Glu	Glu	Trp	
2540						2545					2550					
GAT	ATT	GGT	GAG	CAA	ATG	CTG	CGA	AAT	TTG	TAC	ACT	GAG	ATA	GTG	TAT	7854
Asp	Ile	Gly	Glu	Gln	Met	Leu	Arg	Asn	Leu	Tyr	Thr	Glu	Ile	Val	Tyr	
2555					2560				2565						2570	
ACA	CCA	ATC	CTC	ACA	CCG	GAT	GGT	ACT	ATC	ATT	AAG	AAG	CAT	AAA	GGC	7902
Thr	Pro	Ile	Leu	Thr	Pro	Asp	Gly	Thr	Ile	Ile	Lys	Lys	His	Lys	Gly	
				2575					2580						2585	
AAC	AAT	AGC	GGG	CAA	CCT	TCA	ACA	GTG	GTG	GAC	AAC	ACA	CTC	ATG	GTC	7950
Asn	Asn	Ser	Gly	Gln	Pro	Ser	Thr	Val	Val	Asp	Asn	Thr	Leu	Met	Val	
			2590					2595							2600	
ATT	ATT	GCA	ATG	TTA	TAC	ACA	TGT	GAG	AAG	TGT	GGA	ATC	AAC	AAG	GAA	7998
Ile	Ile	Ala	Met	Leu	Tyr	Thr	Cys	Glu	Lys	Cys	Gly	Ile	Asn	Lys	Glu	
		2605					2610					2615				
GAG	ATT	GTG	TAT	TAC	GTC	AAT	GGC	GAT	GAC	CTA	TTG	ATT	GCC	ATT	CAC	8046
Glu	Ile	Val	Tyr	Tyr	Val	Asn	Gly	Asp	Asp	Leu	Leu	Ile	Ala	Ile	His	
		2620				2625						2630				
CCA	GAT	AAA	GCT	GAG	AGG	TTG	AGT	AGA	TTC	AAA	GAA	TCT	TTC	GGA	GAG	8094
Pro	Asp	Lys	Ala	Glu	Arg	Leu	Ser	Arg	Phe	Lys	Glu	Ser	Phe	Gly	Glu	
					2640					2645					2650	
TTG	GGC	CTG	AAA	TAT	GAA	TTT	GAC	TGT	ACC	ACC	AGG	GAC	AAG	ACA	CAG	8142
Leu	Gly	Leu	Lys	Tyr	Glu	Phe	Asp	Cys	Thr	Thr	Arg	Asp	Lys	Thr	Gln	
			2655					2660							2665	
TTG	TGG	TTC	ATG	TCA	CAC	AGG	GCT	TTG	GAG	AGG	GAT	GGC	ATG	TAT	ATA	8190
Leu	Trp	Phe	Met	Ser	His	Arg	Ala	Leu	Glu	Arg	Asp	Gly	Met	Tyr	Ile	
			2670					2675					2680			
CCA	AAG	CTA	GAA	GAA	GAA	AGG	ATT	GTT	TCT	ATT	TTG	GAA	TGG	GAC	AGA	8238
Pro	Lys	Leu	Glu	Glu	Glu	Arg	Ile	Val	Ser	Ile	Leu	Glu	Trp	Asp	Arg	
			2685				2690					2695				
TCC	AAA	GAG	CCG	TCA	CAT	AGG	CTT	GAA	GCC	ATC	TGT	GCA	TCA	ATG	ATT	8286
Ser	Lys	Glu	Pro	Ser	His	Arg	Leu	Glu	Ala	Ile	Cys	Ala	Ser	Met	Ile	
		2700				2705					2710					
GAA	GCA	TGG	GGT	TAT	GAC	AAG	CTG	GTT	GAA	GAA	ATC	CGC	AAT	TTC	TAT	8334
Glu	Ala	Trp	Gly	Tyr	Asp	Lys	Leu	Val	Glu	Glu	Ile	Arg	Asn	Phe	Tyr	
		2715			2720				2725						2730	
GCA	TGG	GTT	TTG	GAA	CAA	GCG	CCG	TAT	TCA	CAG	CTT	GCA	GAA	GAA	GGA	8382
Ala	Trp	Val	Leu	Glu	Gln	Ala	Pro	Tyr	Ser	Gln	Leu	Ala	Glu	Glu	Gly	
			2735					2740						2745		
AAG	GCG	CCA	TAT	CTG	GCT	GAG	ACT	GCG	CTT	AAG	TTT	TTG	TAC	ACA	TCT	8430
Lys	Ala	Pro	Tyr	Leu	Ala	Glu	Thr	Ala	Leu	Lys	Phe	Leu	Tyr	Thr	Ser	
			2750					2755					2760			
CAG	CAC	GGA	ACA	AAC	TCT	GAG	ATA	GAA	GAG	TAT	TTA	AAA	GTG	TTG	TAT	8478
Gln	His	Gly	Thr	Asn	Ser	Glu	Ile	Glu	Glu	Tyr	Leu	Lys	Val	Leu	Tyr	
		2765				2770						2775				
GAT	TAC	GAT	ATT	CCA	ACG	ACT	GAG	AAT	CTT	TAT	TTT	CAG	AGT	GGC	ACT	8526
Asp	Tyr	Asp	Ile	Pro	Thr	Thr	Glu	Asn	Leu	Tyr	Phe	Gln	Ser	Gly	Thr	
		2780				2785					2790					

FIG. 1

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GTG GAT GCT GGT GCT GAC GCT GGT AAG AAG AAA GAT CAA AAG GAT GAT Val Asp Ala Gly Ala Asp Ala Gly Lys Lys Lys Asp Gln Lys Asp Asp 2795 2800 2805 2810	8574
AAA GTC GCT GAG CAG GCT TCA AAG GAT AGG GAT GTT AAT GCT GGA ACT Lys Val Ala Glu Gln Ala Ser Lys Asp Arg Asp Val Asn Ala Gly Thr 2815 2820 2825	8622
TCA GGA ACA TTC TCA GTT CCA CGA ATA AAT GCT ATG GCC ACA AAA CTT Ser Gly Thr Phe Ser Val Pro Arg Ile Asn Ala Met Ala Thr Lys Leu 2830 2835 2840	8670
CAA TAT CCA AGG ATG AGG GGA GAG GTG GTT GTA AAC TTG AAT CAC CTT Gln Tyr Pro Arg Met Arg Gly Glu Val Val Val Asn Leu Asn His Leu 2845 2850 2855	8718
TTA GGA TAC AAG CCA CAG CAA ATT GAT TTG TCA AAT GCT CGA GCC ACA Leu Gly Tyr Lys Pro Gln Gln Ile Asp Leu Ser Asn Ala Arg Ala Thr 2860 2865 2870	8766
CAT GAG CAG TTT GCC GCG TGG CAT CAG GCA GTG ATG ACA GCC TAT GGA His Glu Gln Phe Ala Ala Trp His Gln Ala Val Met Thr Ala Tyr Gly 2875 2880 2885 2890	8814
GTG AAT GAA GAG CAA ATG AAA ATA TTG CTA AAT GGA TTT ATG GTG TGG Val Asn Glu Glu Gln Met Lys Ile Leu Leu Asn Gly Phe Met Val Trp 2895 2900 2905	8862
TGC ATA GAA AAT GGG ACT TCC CCA AAT TTG AAC GGA ACT TGG GTT ATG Cys Ile Glu Asn Gly Thr Ser Pro Asn Leu Asn Gly Thr Trp Val Met 2910 2915 2920	8910
ATG GAT GGT GAG GAT CAA GTT TCA TAC CCG CTG AAA CCA ATG GTT GAA Met Asp Gly Glu Asp Gln Val Ser Tyr Pro Leu Lys Pro Met Val Glu 2925 2930 2935	8958
AAC GCG CAG CCA ACA CTG AGG CAA ATT ATG ACA CAC TTC AGT GAC CTG Asn Ala Gln Pro Thr Leu Arg Gln Ile Met Thr His Phe Ser Asp Leu 2940 2945 2950	9006
GCT GAA GCG TAT ATT GAG ATG AGG AAT AGG GAG CGA CCA TAC ATG CCT Ala Glu Ala Tyr Ile Glu Met Arg Asn Arg Glu Arg Pro Tyr Met Pro 2955 2960 2965 2970	9054
AGG TAT GGT CTA CAG AGA AAC ATT ACA GAC ATG AGT TTG TCA CGC TAT Arg Tyr Gly Leu Gln Arg Asn Ile Thr Asp Met Ser Leu Ser Arg Tyr 2975 2980 2985	9102
GCG TTC GAC TTC TAT GAG CTA ACT TCA AAA ACA CCT GTT AGA GCG AGG Ala Phe Asp Phe Tyr Glu Leu Thr Ser Lys Thr Pro Val Arg Ala Arg 2990 2995 3000	9150
GAG GCG CAT ATG CAA ATG AAA GCT GCT GCA GTA CGA AAC AGT GGA ACT Glu Ala His Met Gln Met Lys Ala Ala Ala Val Arg Asn Ser Gly Thr 3005 3010 3015	9198
AGG TTA TTT GGT CTT GAT GGC AAC GTG GGT ACT GCA GAG GAA GAC ACT Arg Leu Phe Gly Leu Asp Gly Asn Val Gly Thr Ala Glu Glu Asp Thr 3020 3025 3030	9246
GAA CGG CAC ACA GCG CAC GAT GTG AAC CGT AAC ATG CAC ACA CTA TTA Glu Arg His Thr Ala His Asp Val Asn Arg Asn Met His Thr Leu Leu 3035 3040 3045 3050	9294

FIG. 1

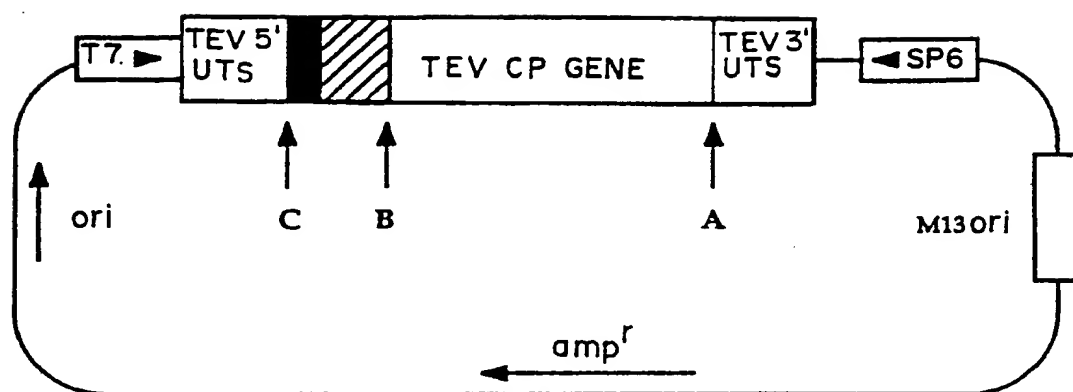
SUBSTITUTE SHEET

GGG GTC CGC CAG TGA TAGTTTCTGC GTGTCTTTGC TTTCCGCTTT TAAGCTTATT	9349
Gly Val Arg Gln	
GTAATATATA TGAATAGCTA TTCACAGTGG GACTTGGTCT TGTGTTGAAT AGTATCTTAT	9409
ATATTTTAAT ATGTCTTATT AGTCTCATT CTTAGGCGAA CGACAAAGTG AGGTCACCTC	9469
GGTCTAATTC TCCTATGTAG TGCGAG	9495

FIG. 1

SUBSTITUTE SHEET

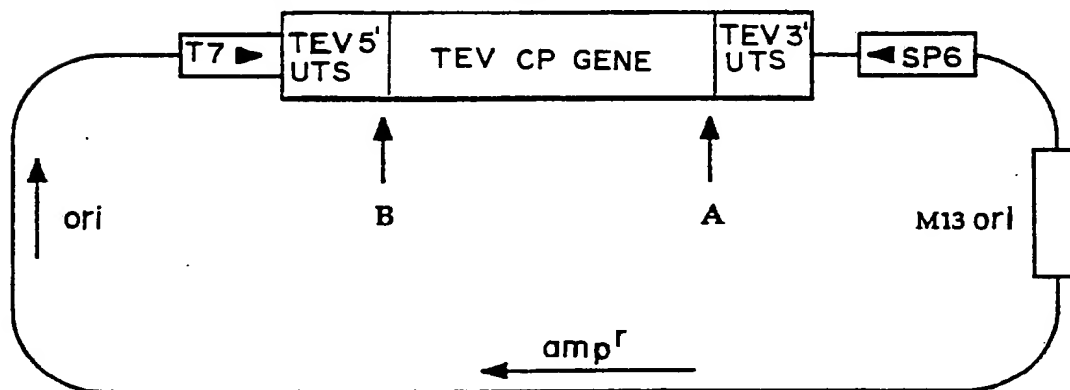
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pTL 37/8595

1. GENERATE BamHI SITE AT A (nt 9312-9317)
2. GENERATE NcoI SITE AT B (nt 8516-8521)
3. GENERATE BamHI SITE (nt 133-138) NcoI SITE (nt 143-148) AND DEOXYADENYLATE RESIDUE (at nt 142) at C.

DIGEST WITH NcoI  
 REMOVE TEV NUCLEOTIDES 143-200/8462-8516  
 (FLANKED BY SITES B AND C) AND RELIGATE.



pTC:FL

**FIG. 2**

TEV Coat Protein Gene Constructs Inserted  
into *Nicotiana tabacum* cv. Burley 49

Plant Line

Product in  
Transgenic Plants

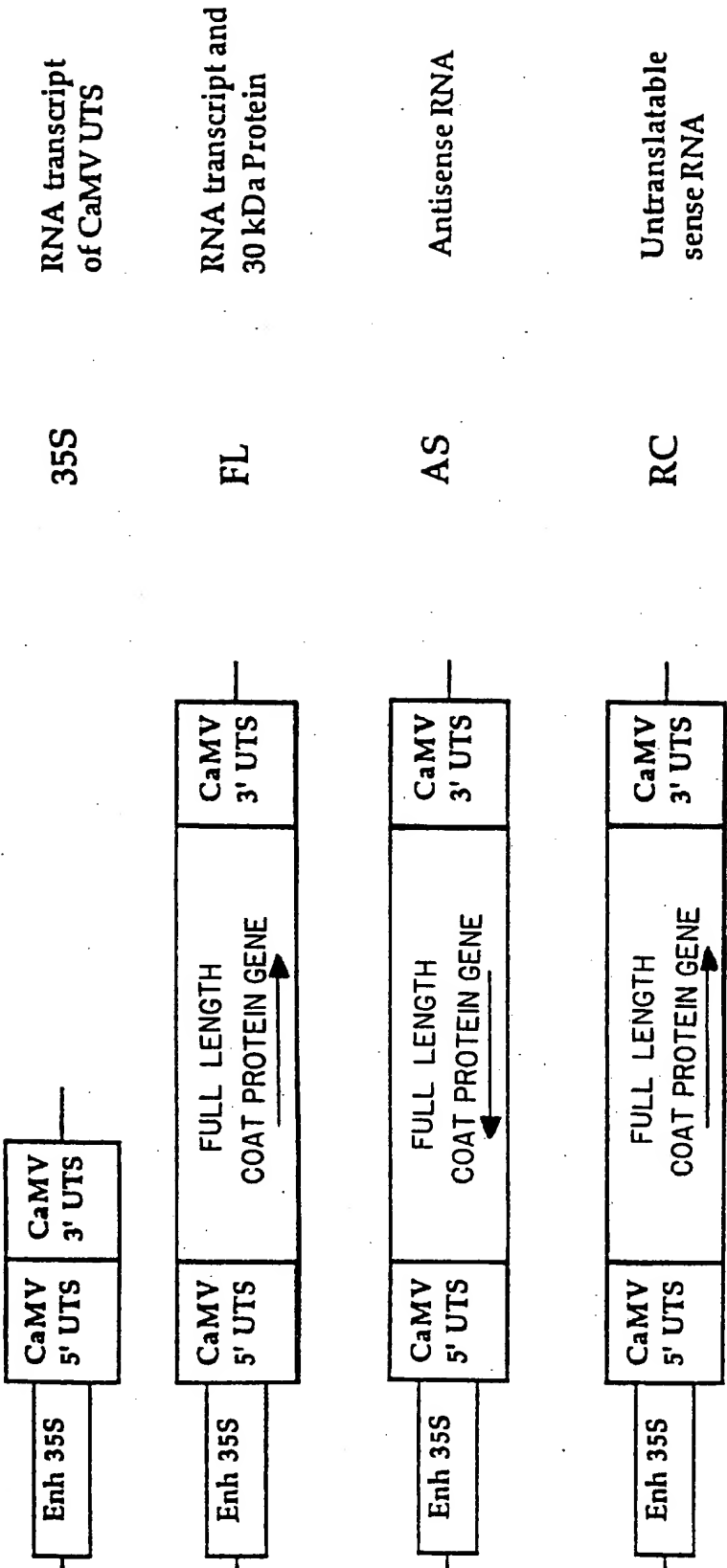
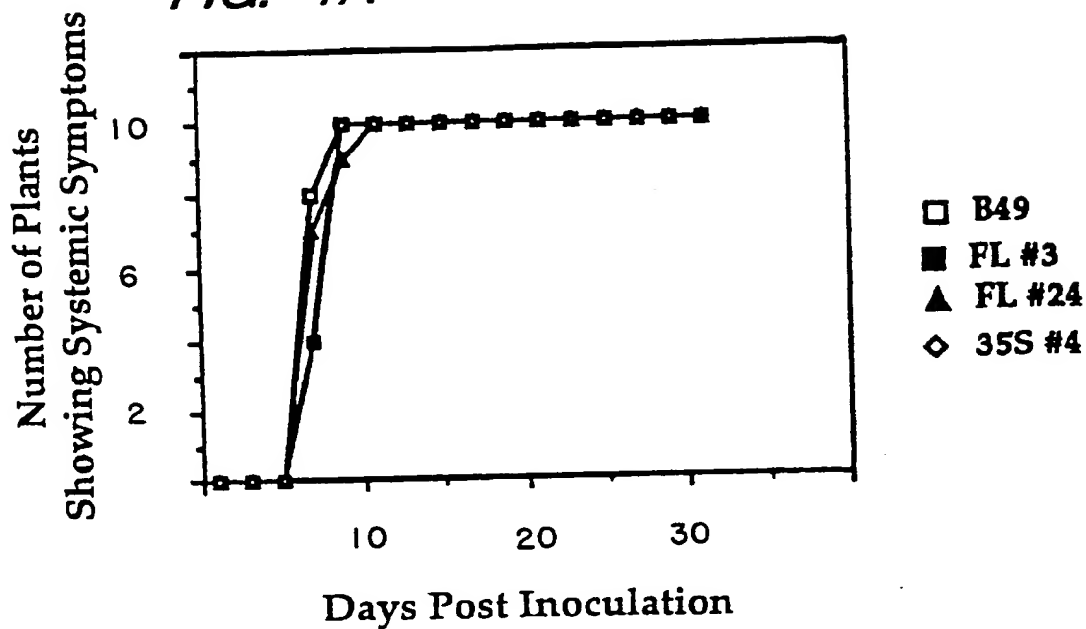
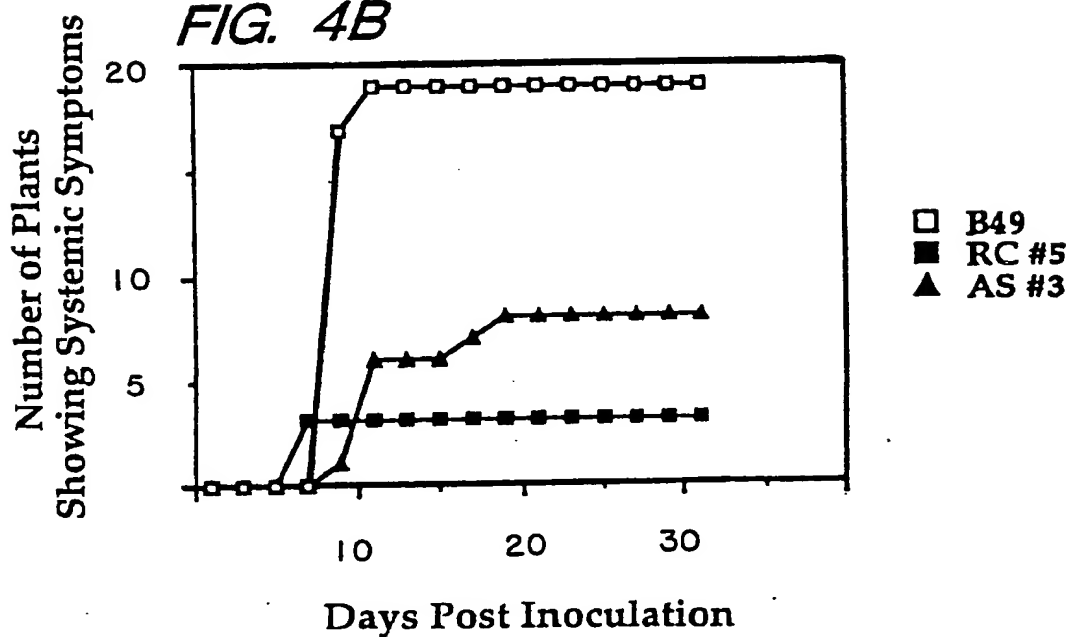


FIG. 3

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**FIG. 4A****FIG. 4B**

## INTERNATIONAL SEARCH REPORT

International Application No.  
PCT/US93/01544**A. CLASSIFICATION OF SUBJECT MATTER**IPC(5) : C12N 1/21, 5/10, 15/33, 15/82; C07H 21/04; A01H 5/00  
US CL : 435/172.3, 240.4, 252.3, 320.1; 536/23.72; 800/205

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/172.3, 240.4, 252.3, 320.1; 536/23.72; 800/205

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, DIALOG,  
search terms: virus or viral, untranslat?, resistan?**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	Molecular Plant-Microbe Interactions, Volume 5, No. 2, issued March 1992, Lindbo et al, "Pathogen-derived resistance to a potyvirus: immune and resistant phenotypes in transgenic tobacco expressing altered forms of a potyvirus coat protein nucleotide sequence", pages 144-153, see entire document.	1-27
X,P	Virology, Volume 189, No. 2, issued August 1992, Lindbo et al, "Untranslatable transcripts of the tobacco etch virus coat protein gene sequence can interfere with tobacco etch virus replication in transgenic plants and protoplasts", pages 725-733, see entire document.	1-27



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be part of particular relevance	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E" earlier document published on or after the international filing date	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"A" document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

03 May 1993

Date of mailing of the international search report

26 MAY 1993

Name and mailing address of the ISA/US  
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Box PCT  
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## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US93/01544

## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
<del>X</del> Y	Molecular Plant-Microbe Interactions, Volume 4, No. 3, issued May 1991, Kawchuk et al, "Sense and antisense RNA-mediated resistance to potato leafroll virus in russet burbank potato plants", pages 247-253, see entire document.	1, 6-8, 13, <u>18, 22-23</u> 2-5, 9-12, 14-17, 19-21, 24-27
<del>X</del> Y	Plant Molecular Biology, Volume 17, issued 1991, van der Wilk et al, "Expression of the potato leafroll luteovirus coat protein gene in transgenic potato plants inhibits viral infection", pages 431-439, see entire document.	1, 6-8, 13, <u>18, 22-23</u> 2-5, 9-12, 14-17, 19-21, 24-27
<del>X</del> Y	Journal of General Virology, Volume 72, issued August 1991, Marsh et al, "Artificial defective interfering RNAs derived from brome mosaic virus", pages 1787-1792, see entire document.	1, 6-8, 13, <u>18, 22-23</u> 2-5, 9-12, 14-17, 19-21, 24-27
<del>X</del> Y	Proceedings of the National Academy of Sciences USA, Volume 88, issued August 1991, Day et al, "Expression of an antisense viral gene in transgenic tobacco confers resistance to the DNA virus tomato golden mosaic virus", pages 6721-6725, see entire document.	1, 6-8, 13, <u>18, 22-23</u> 2-5, 9-12, 14-17, 19-21, 24-27
X	Virology, Volume 175, issued 1990, Powell et al, "Protection against tobacco mosaic virus infection in transgenic plants requires accumulation of coat protein rather than coat protein RNA sequences", pages 124-130, see entire document.	1, 6-8, 13, 18, 22-23
Y	Virology, Volume 154, issued 1986, Allison et al, "The nucleotide sequence of the coding region of tobacco etch virus genomic RNA: evidence for the synthesis of a single polyprotein", pages 9-20, see entire document.	2-5, 9-12, 14-17, 19-21, 24-27
Y	Trends in Genetics, Volume 5, No. 2, issued February 1989, Baulcombe, "Strategies for virus resistance in plants", pages 56-60, see entire document.	2-5, 9-12, 14-17, 19-21, 24-27